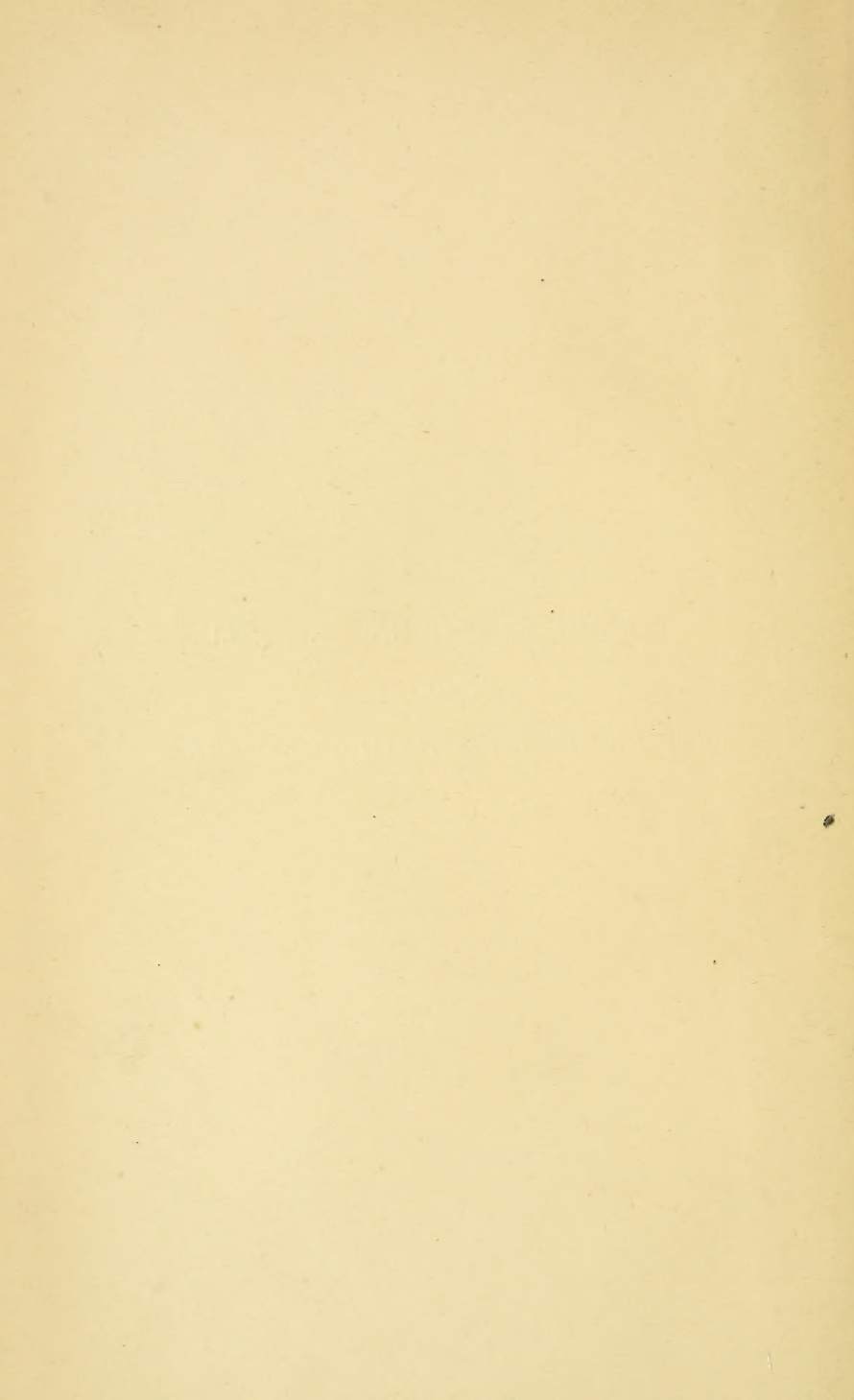


THE INTERNATIONAL JOURNAL
OF
MICROSCOPY AND NATURAL SCIENCE.



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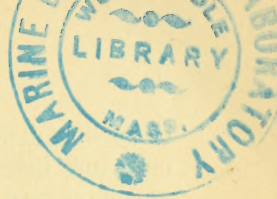
THE JOURNAL OF
THE POSTAL MICROSCOPICAL SOCIETY.

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VOL. III. THIRD SERIES.
VOL. XII. OLD SERIES.

London :
BAILLIERE, TINDALL, & COX, 20 KING WILLIAM ST., STRAND.
BATH : 1 CAMBRIDGE PLACE.
1893.



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*"Knowledge is not given us to keep, but to impart; its worth
is lost in concealment."*

[The Editor does not hold himself responsible for the views of
the authors of the papers published.]

Presidential Address.

Polarised Light and its Applications to the Microscope.

PRESIDENTIAL ADDRESS BY G. H. BRYAN, M.A.

PART I.



ON a recent occasion, Lord Kelvin (or rather Sir William Thomson, for he had not then risen to the rank of a "scientific peer"), in the course of a highly interesting lecture on "Motivity," remarked, with reference to the Second Law of Thermodynamics, that he would not attempt to explain the law that evening, "for," as he said, "it could not be explained satisfactorily with less than six hours of tutorial instruction." Now, the same thing is true of polarised light, but with this difference, that to explain *that* thoroughly, about twelve hours of "tutorial instruction" would be about the minimum. In the short space at my disposal, it will not

be possible to give more than the barest outline of the subject ; but I trust that every one of my readers will derive a fairly clear notion of how it is that we see certain appearances with a polariscope.

Every good microscope for general use is now provided with a polariscope, so that the pretty colours in crystals, the crosses on starch grains, the appearances in a section of rhinoceros horn and the colours seen in plaited horse-hair under the polariscope, both with and without selenite, are well known. But life is too short for every microscopist to become at the same time a physical optician, a botanist, a chemist, a geologist, a physiologist, an entomologist, a bacteriologist, and a diatomist ; and my purpose now is to try and explain a little about polarised light to those who have not made physical optics their speciality. I shall not attempt to explain any phenomena except those which relate to the construction of a polariscope and the appearances which it produces in *microscopic objects*.

What is polarised light? Mr. Spottiswoode, in his valuable little book, says, by way of introduction :—" Light is said to be polarised when it presents certain peculiarities, hereafter to be described, which it is not generally found to possess !" These peculiarities he goes on to describe, but for our present purpose it will be better to commence by asking the question, " What is light ? "

Sir Isaac Newton was the first who tried to answer this question, but his " corpuscular theory," while accounting fairly well for some of the simpler optical effects, presented such difficulties when applied to polarisation and other phenomena, that it had to be abandoned in favour of the wave theory, due to Huyghens and Fresnel, and which is now universally accepted as the basis of modern optics.

The Undulatory Theory.—According to this theory, light consists in a series of vibrations, which are propagated through space in the form of waves. In a light-wave the vibratory motion is in a direction *perpendicular* to that in which the light is travelling. To illustrate this, shake any part of a piece of string (stretched horizontally between two fixed points) from side to side. You will

see that the vibration is transmitted along the string, each point of the string in turn taking up the motion and swinging from side to side. Fig. 1 also shows a wave of this kind in eight different stages of its progress. If we look at the string as a whole, we shall see the appearance of waves travelling *along* it, although if we look at any particular knot we see that it moves backwards and forwards *perpendicularly* to the string. We here have vibration going on in a direction perpendicular to the line along which it is being propagated.

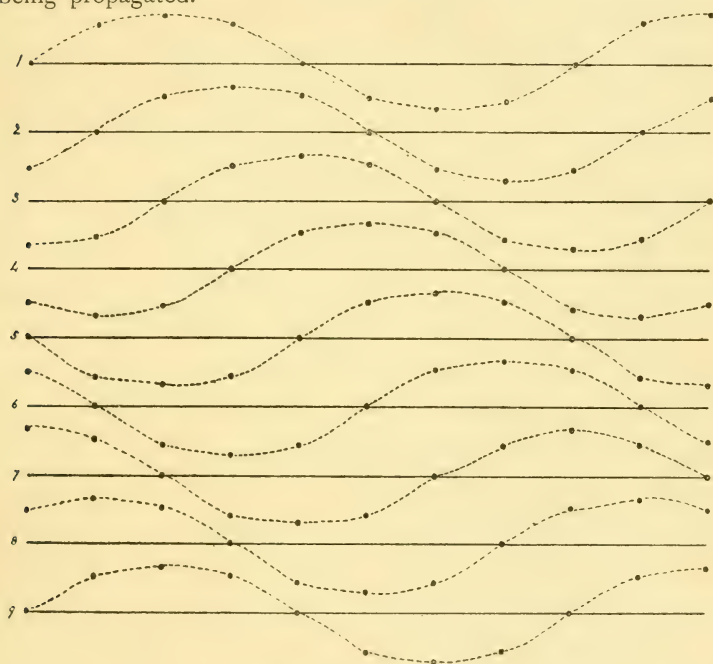


Fig. 1.—LIGHT-WAVES.

If light consists of vibrations such as these, there must be something to vibrate. We can understand a piece of jelly or india-rubber vibrating from side to side in this way; but nothing of the nature of a fluid such as air could do so, much less a vacuum, and light *can* travel through a vacuum. To account for light-waves, it is customary to suppose space to be filled with a kind of substance called the *ether*, which allows ordinary matter

to move about in it perfectly freely and without hindrance, and yet behaves like a mass of jelly in the matter of transmitting these waves of light. Such a jelly seems at first sight rather hard to believe, but according to modern views electrical and magnetic phenomena also require the existence of an ether, and, what is most remarkable, the same ether that will account for electricity and magnetism will also account for light. Indeed, the recent researches of Prof. Hertz and numerous others following in his footsteps have shown that electrical waves can be propagated, possessing properties exactly analogous to those of light-waves, and from these and other experiments we have abundant evidence that light-waves are in reality electrical oscillations.

But whether we regard the ether as a material jelly-like solid, or adopt the theory that light is an electric phenomenon, the fact always remains that *the light-vibrations take place in a direction transverse to the line along which the light is travelling*, and this is the only point upon which I wish now to lay stress as affecting the phenomena of polarisation.

Polarised Light.—If we take a string stretched horizontally across the room, we can make it vibrate transversely in any number of different directions. We can shake its end backwards and forwards horizontally, when the whole will vibrate in a horizontal plane, or we can shake it up and down and make the string vibrate vertically, or by shaking it in any slanting direction we can make it vibrate in any other plane. In just the same way, if we suppose a beam of light travelling through the ether in the direction of the string, the particles of ether might vibrate horizontally or up and down, or in any of the other directions. In this case the plane of vibration of the ether, or the corresponding plane in which the string vibrated, is called the *plane of polarisation* and the light is said to be *plane polarised*. By revolving the end of the string in a circle, every other point will be made to revolve in a circle, and this kind of motion may be taken as representing a beam of what is called *circularly polarised* light.

Finally, if we shake the string about indiscriminately in different directions, we get a more general kind of vibration, and this represents the kind of motion that goes on in the ether when a

beam of ordinary light not polarised in any particular way is propagated through it.

To polarise a beam of ordinary light, then, it is necessary to have some arrangement which will cause the vibrations of the ether particles to be restricted to one plane by destroying any vibratory motion they may have in a direction perpendicular to that plane.

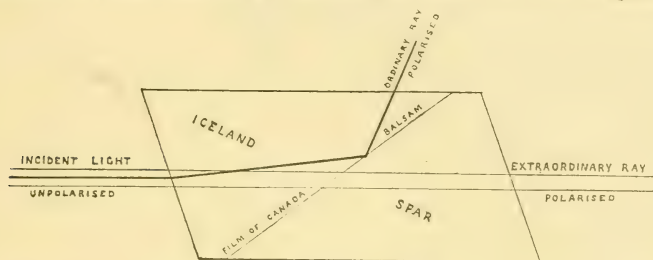


Fig. 2.—NICOL'S PRISM.

Polarisation by Double Refraction.—If we take a piece of Iceland spar and lay it over a piece of black paper in which a small pinhole has been made, the pinhole appears doubled. If the spar is laid on a piece of paper with writing on it, the writing similarly appears doubled. This shows that the spar divides every beam of light into two, which pass through it in different directions. The beams of light which are thus separated by the crystal are polarised in two perpendicular directions. If we can get rid of one of the two beams, we shall thus be able to get a beam of polarised light.

This is done by cutting the crystal of spar in halves and then joining the two halves together with Canada balsam (Fig. 2). One of the two polarised beams in the crystal (called the *ordinary ray*) strikes the balsam surface so obliquely that it cannot pass through, but is totally reflected out of the way. The other beam (called the *extraordinary ray*) strikes the surface less obliquely, and most of it is transmitted through the balsam and emerges at the opposite surface of the crystal.

This arrangement is called a *Nicol's prism*, and the polariscope of a microscope consists essentially of two Nicol's prisms—one fixed below and the other fixed above the object to be examined. The former is called the *polariser* and the latter is called the *analyser*.

If the two Nicol's prisms—the polariser and the analyser—be placed parallel to each other, the components of the light, which are transmitted by the polariser, are polarised in the right direction to be transmitted by the analyser, and we get a bright field of view. But if the polariser is turned through a right angle, the plane of polarisation of the light transmitted by it will be that of the more oblique beam, which is totally reflected by the balsam in the analyser, so that the whole of the light will now be extinguished and the background will be dark. In this case the Nicol's prisms are said to be *crossed*.

If any object be now placed on the stage of the microscope, and if it be of such a nature that the light continues polarised in the same plane after passing through it, this light is still arrested by the analyser and the object remains invisible. If, therefore, we define a *polariscopic object* as one which is visible when the polariscope gives a dark ground, we see that polariscopic objects must possess the property of affecting a beam of plane polarised light in such a way that after passing through them it is no longer polarised in the same plane.

VASCULAR TISSUES.—I should be glad of a short list of widely distributed plants, which are most suitable for showing spiral and annular vessels, pitted ducts, etc. In one of the editions of Carpenter's "Revelations," there is a woodcut of a longitudinal section of what he calls "Italian Reed," which shows very clearly and beautifully, in one bundle, all the above forms of vessels and ducts. I should be glad to know more about the reed in question. If not a native plant, as the name would suggest, how may it be procured? An answer by a botanical reader would much oblige.

R. W. A.

KING'S FLUID.—What is known in America as King's fluid for algæ is really Petit's, but as the Rev. Mr. King introduced it, it was called by his name. The formula is as follows:—Take Camphor Water, 50 grms.; Distilled Water, 50 grms.; Glacial Acetic Acid, 0·50 grms.; Chloride of Copper Crystals, 0·20 grms.; Nitrate of Copper, 0·20 grms.

V. A. L.

Soaking Tissues and Sections of Tissues in Water.

BY J. W. PLAXTON (Kingston, Jamaica).

HAS anyone but myself been led astray by this practice? Or has anyone made use of it in the manner I should be inclined to do if once more I were to have a class to teach?

Soaking in water "for a night" or for "from twelve to twenty-four hours" is enjoined as preliminary to embedding spirit-hardened tissues in gum and before cutting sections. I do not myself embed in gum or use a freezing microtome, but usually embed in paraffin; I have not, therefore, suffered by soaking the tissue in bulk, but I have on two occasions left sections in water overnight, and stained and mounted the following morning.

In the first case I was engaged in searching for a possible microphytic ferment in the edible arillus of the fruit of the Akee (*Cupania edulis*), which, under ordinary circumstances, is most nutritious, and a very good substitute indeed for Yorkshire pudding; but, in this case under investigation, proving poisonous, as it sometimes will, had literally exterminated a family:—five human beings, their cat, and their dog. The sections in the second instance were cut from the skin of a case of leprosy.

Hundreds of the *unsoaked* sections of the Akee had shown themselves absolutely sterile. The *unsoaked* sections of leprosy had shown the bacilli of that disease most magnificently when stained in magenta. When, however, I came to examine the *soaked* sections of Akee, to my transitory astonishment bacteria were numerous in them and beautifully displayed. In like manner, my *soaked* sections of leprotic material, which I had, fortunately, stained in gentian violet, though they did not show a single bacillus of leprosy stained, showed strange bacteria scattered through the sections in all the vivid beauty of poppies

(violet poppies!) in a corn-field. In both these cases the microphytes were palpably, almost, what we, ancients, would have recognised as *Bacterium termo*. Finally, a friend who uses the gum-embedding method brought me a few recently made and mounted sections of a tumour. In them bacteria are easily demonstrable. I knew that the intruding organisms could only have made a home in the tissue during the preliminaries of mounting.

Of course, the temperature of my work-room, and that of my friend, here, in Jamaica, was a tropic temperature of, say, 80° F., but the temperature of an English summer may well approximate to this; and, hence, prove equally favourable to the development of saprophytic organisms, and to the delusion of a Bacteriologist. I may here say that, taking warning by my first misleading, I was alive to the danger afterwards, and did, as a precaution, before leaving work for the day, float a lump of camphor on the water with the sections.

By a teacher the cultivation, for a few hours, of organisms in sections in this way could be made good use of as a first lesson in the microscopy of microphytic organisms in tissues. There is no huddling together; the bacteria are superficial, scattered, solitary, or in small groups—discrete, as the doctors would say of pustules; and they in every way lend themselves for observation and study.

Perhaps some may benefit by the warning; others by a useful hint.

A FIFTH SATELLITE of Jupiter was discovered by Professor Barnard, of the Leek Observatory, Sept. 12, 1892, and had been observed by him to October 17th on seven successive nights. It was also seen by Mr. Reed, at Princetown, on October 10th, with a 23-inch telescope. It was a star of the thirteenth magnitude. From three hundred micrometric observations by Prof. Barnard, and the observation at Princetown, a period has been approximately deduced of eleven hours and fifty-seven minutes.

Concerning the Rules and Appliances of Reichert's Hæmometer.*

BY FREDERICK GAERTNER, M.D., PITTSBURG, PA.

THIS apparatus is designed to ascertain the amount of hæmoglobin in either a diseased or a normal condition of the blood. It was devised by Prof. E. von Fleischl, and patented by Carl Reichert, of Vienna. (See Fig. 3). This

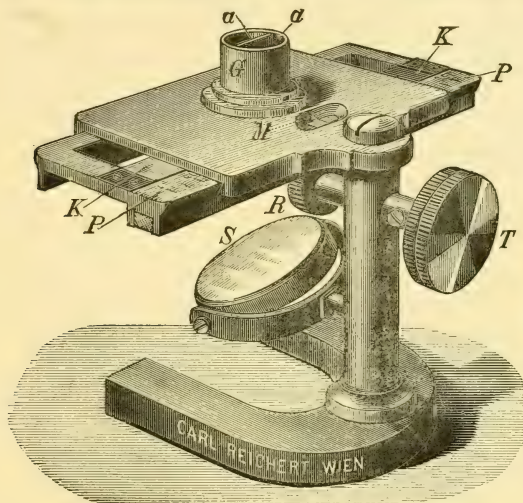


Fig. 3.—REICHERT'S HÆMOMETER.

little instrument, the Hæmometer, is the result of a need felt by physicians and scientists of having an instrument which will give a quantitative judgment (analysis) of the value and function of the hæmoglobin in the circulating blood. It was further necessitated by the inapplicability of the methods thus far prescribed for this purpose to the cases encountered by physicians; and, finally, it arose from the hope of advancing our physiological and clinical knowledge by rating the per cent. of hæmoglobin in diseased human blood.

The Hæmometer cannot be used either by daylight or by the electric light, and only by the light of oil lamps, candles, and gas.

* Read before the Iron City Microscopical Society.



Every examination of blood by means of the Hæmometer must consist of these three operations:—1.—To obtain and measure the blood. 2.—To dissolve it in water, and to fill the instrument with this solution. 3.—To arrange the instrument and read the results.

This apparatus consists of a small and simply constructed horse-shoe base, composed of a foot, column, mirror, and table. Beneath the table is a frame which bears the glass wedge, *K*, the latter being propelled by the milled-head screw, *R*. Upon the table is a cylindrical vessel, *G*, the one-half of which (*a*) is filled with blood which has been diluted with water, so as to be examined. The other half (*a*) is filled with pure spring water, after a tube, whose capacity has been exactly gauged, has itself been filled with blood by capillary action. It is brought into the half of the vessel at *a*, where the blood contained in the tube dissolves in the water until it becomes a perfectly transparent liquid. By the optical conditions of the apparatus it becomes possible, under an illumination of oil lamps, candles, or gas light, to find a position of the glass wedge, *K*, at which the colour and brightness of every such blood solution is exactly the same. This point is sought by moving the wedge backwards and forwards by means of the micrometric screw, *T*, and by giving the reflector a definite position, *S*.

Upon the frame which surrounds the wedge, a scale, *P*, is engraved, a part of which is visible through the aperture at *M*. This gives exact results in percentage of the amount of hæmoglobin in a certain blood solution. There is also a stationary index line on the side of the aperture, *M*, which points also to the discovered amount on the scale.

This Hæmometer presents the following advantages:—

- 1.—Easy and convenient management of the apparatus.
- 2.—Rapid and direct results in percentage regarding the degree of normal hæmoglobin.
- 3.—The small quantity of blood, only a drop, required for the examination.

It is best to take the blood from the tip of the left middle finger.

After the skin has been thoroughly washed and carefully dried,

and without any preceding compression, or binding of the finger, as is usually done, it should be wounded by a slight prick with a sharp needle. Then by a slight pressure above the little wound a drop of blood is secured. This drop of blood is taken up with one of the open ends of an automatic blood pipette, a small capillary tube about 8 mm. in length, bound about in the centre by a tiny wire, and of definite capacity ($6\frac{1}{2}$ cubic mm.). The filling of the automatic blood pipette is considerably facilitated and accelerated by holding it horizontally, instead of perpendicularly ; that is, it is dipped sideways into a drop of blood.

Since every trace of blood that clings to the exterior of the tube is to be considered a serious defect, it is necessary to smear the pipette with something of a fatty nature. This is best done by keeping it in a leather case, lubricated with tallow. As soon as the pipette is full the outer surface should be carefully examined. If a speck of blood is found there, it must instantly be removed, or before it has time to dry. This is done by means of a strip of filtering paper or absorbent cotton. The blood is then much more fully and easily absorbed when the exterior of the glass is coated with an oleaginous substance. Care should be taken that the column of blood ends at both extremities, on the same level with the glass tubes, and neither with retiring nor with bulging, but with even extremities. If it should be necessary to use filtering paper or wadding to remove the blood from the exterior of the pipette, care should be taken that these substances do not approach too closely to the extremities of the blood column, in order to avoid a meniscus.

Even before these instructions are carried out, the various parts of the Hæmometer should be examined to insure perfect cleanliness, and a perfect condition of the apparatus. The component parts may then be arranged. The frame upon which the red glass wedge reposes must be joined to the wing on the lower side of the table slab, through which it finds its guidance. Moreover, the comparing vessel must be inserted into the opening designed for it in the table slab, and so placed that the projection of the vessel, as observed from above, may coincide with the visible part of the free wedge lying beneath.

Both halves of the comparing vessel must be filled with dis-

tilled or pure spring water. The half above the wedge, called "wedge half," is completely filled with water from the pipette, so that the smooth surface which it forms above may be perfectly level, forming neither a positive nor a negative meniscus. The other, the blood half, is also filled with water from the pipette, but only to about one-fifth, or at most, one-fourth of its capacity. When this is done, the pipette out of which the vessel has been filled, and which still contains a sufficient quantity of water to complete the filling of the blood half, should be placed in a horizontal position—*i.e.*, upon the brim of a goblet, so that the water will not flow out of it.

The pipette having been filled with blood, it should be brought (in a horizontal position) under the water in the blood half of the comparing vessel, when the little wire should be leaned against the upper edge of the vessel, but not against the straight edge of the partition wall, nor in either one of the corners at the end of the same, but against the middle point of the curved edge of the blood half. In this manner the little tube with the blood is made to lie in the centre of the rectilinear chamber, which the partition wall touches at the bottom of the vessel.

The blood pipette should not be permitted to remain quietly in that position under water, but a gentle motion should be imparted by a judicious guidance of the little wire to which the pipette is fastened; that is, the little tube should be moved backward and forward along its own axis as far as the dimensions permit, and in this manner be moved to and fro over its fluid contents.

It is easily seen that these movements are directed to produce a speedy solution of the contents of the tube with the surrounding fluid. It is also readily seen how important it is that no time be wasted in the proceedings following the taking of the blood, but rather that all should be arranged as quickly as possible without neglecting carefulness and exactness of execution. For the rest, the caution not to work more slowly than necessary, refers only to the manipulations. These motions are so easy and simple that even an unskilled hand will need not more than one minute for their execution. That much of time may pass without endangering the result in determining the amount of hæmoglobin.

All depends upon the blood being mixed with a certain quantity of water sufficient to dissolve it before it coagulates. The shorter and broader the capillary, the more rapidly the blood in the graduating capillary will mix with the surrounding water. The volume of blood used for measuring will be determined with greater exactness, the longer and narrower the graduating capillary is. The most advantageous length and breadth of the blood pipette is that which permits a rapid mixing of the blood and water with a sufficient exactness in determining the volume. My experience permits me to give a warning against the use of blood pipettes, however well gauged, which are shorter than 7 mm., or longer than 10 mm. Moreover, the edge of the blood pipette must be rounded, must be allowed to shape itself in the flame, but neither of the openings should be contracted nor narrowed.

As soon as most of the contents of the blood pipette has entered the water, the pipette should be withdrawn by the little wire and held in a vertical position over the same, so that the lower opening in the tube in the centre of the blood half of the comparing vessel may be suspended several millimeters above the surface of the liquid. Then, with the other hand, seize the drop-pipette, which has already been filled with water, and allow drop after drop to enter the upper end of the blood capillary. By this means not only the contents of the blood capillary, even to the very last traces of blood in the comparing vessel, are cleansed from it, but the traces of blood clinging to the surface of the capillary, and which were lifted from the comparing vessel, are again washed back.

If the drops which have detached themselves from the lower end of the graduating capillary are observed, it may be seen how rapidly the blood drops disappear, and how clear even the fifth or sixth of these drops is. This is also shown under a careful examination by a graduating tube, perfectly clean both within and without, perfectly smooth, and filled as well as washed with clear water. Care must also be taken that no concretions or foreign substances be on or between the coils of the wire which winds about the blood pipette and serves as a handle. Only when all is declared perfectly clean and free from blood, may the blood pipette be wholly removed from the comparing vessel.

The blood half of the comparing vessel, after the graduating tube has been rinsed, should not be much more than half full, never more than three-quarters full of the liquid, first, in order to make a thorough mixing of the contents possible, and second, in order to permit of a last stratum of water above the blood solution. This portion of water renders the overflowing of the partition wall an immaterial instead of a ruinous occurrence. The liquid in the blood half may now be moved with perfect freedom, a thin wire being used to stir it. In the absence of a wire, the handle of a blood pipette may be used ; but in this case the loop which forms the end is an inconvenience, since it prevents the wire from reaching the corners at the bottom of the vessel. And exactly these corners, as well as the angles formed by the bottom and the walls, as also those formed by the partition wall and the mantle of the half cylinder, are the favourite sites of very concentrated parts of the solution. The particles of blood may be so slightly dissolved that no complete dissolution of the hæmoglobin in the water, and even no perfect destruction of the stromata of the red blood cells has taken place in order to secure the hæmoglobin in the solution, in consequence of which the liquid appears turbid. The angles and corners are to be noticed especially, and should be continually observed until neither inequality of colour in the liquid in the blood half of the vessel nor the slightest turbidness can be detected. This of course takes place while the light shines through it, since the vessel has already been set into the instrument (Hæmometer).

When these things have all been arranged, it is time to proceed to the filling of the blood half of the comparing vessel. It is not worth while to rinse back into the vessel the very small portion of the blood solution which clings yet to the end of the wire used to stir it. Pure water from the pipette is then dropped into the blood solution, care being taken that the liquid in the vessel is disturbed as little as possible. With a little practice it may be risked to allow the last quantity of water to flow in, instead of being dropped, while the end of the pipette is dipped slightly beneath the surface of the liquid. The blood half, and also the wedge half, should be filled to the level of the rim, so that no meniscus may occur, but the liquid in both halves may have a

common, absolutely level surface. Only in this case does the partition wall appear in the projection as a parallel limited black stripe, of a thickness corresponding to that of the partition wall. If the liquid in either half, or in both halves, has a meniscus (positive or negative), the dividing line appears distorted, widened in the centre or at both ends, cut by fine glistening white lines, also widened and following the line of the rim in several bands. In a similar manner a coloured field, covered by a meniscus, semi-circular in the interior, and a distortion of the boundary with a contraction of the coloured surface brought forward for comparison is discovered ; although in a lesser degree, this is nevertheless still perceptible just as is the distortion which the picture of the partition wall suffers in consequence of a meniscus. This also affects the exactness and the reliability of the final result. The simplest method of avoiding this defect arising from the presence of the meniscus, is to bestow the requisite amount of attention and care in procuring a perfectly level surface of the fluids in each half of the vessel. Although this task may be disagreeable, it should not be called difficult, since circumstances permit an approach to this end from both sides, and also since the transgression of the proper limit does no great injury. This of course is obvious in regard to the wedge half ; for the blood half the same holds good according to what has already been said. Proceed with the same care in case withdrawal of the surplus liquid is necessary from the blood half. That is needed in adding the last portion of water to this half, as every current may lead to a mixing of the upper and lower layers of water. This surplus of water may be removed either by means of thin glass capillaries or by filtering paper. In either case avoid dipping too deep into the water. The wetting and overflowing of the partition wall may be avoided, when this edge has been greased beforehand.

A second method of eliminating the meniscus presupposes the fulfilment of the instructions given above. This method provides purposely a distinct meniscus for each half, or in case of the overflowing of the partition wall, which is here very probable, fills it until the whole surface forms a convex meniscus. Then place a small cover-glass over the opening of the vessel that no air bubble may be inclosed and without allowing the upper side of

the cover to become wet. It is also necessary to avoid any approach to a stronger current in laying on the cover-glass just as one would reasonably regard the course of an unexpected current.

In the examination of human blood, notwithstanding the considerable quantity added, it is only on very rare occasions that merely and imperfect dissolution of the elements contained in the blood takes place, and in consequence of which there is a certain turbidness of the liquid, so that a physician in his practice will scarcely ever find himself disturbed by this annoyance. On the contrary, in the examination of animal blood, where red blood cells sometimes carry granules, one must be all the better prepared for an imperfect solution and a persistent turbidness in the water. In all such cases the rule of Mr. Leichtenstein is to add a minimum quantity of caustic alkali. This is an excellent rule. Indeed, this investigator praises the effectiveness of fixed alkalies in almost imperceptible doses in every case of protracted turbidness of a stronger and more of a leuchæmic conditions of human blood. By this he refers to a pathological condition, where there is a decided increase of colourless (white) blood cells, and to the great resistance of the same to the effect of water. I know from experience only the clearing effect of this method in thinning blood whose turbidity is the result of the resistance of the granule conveying red blood corpuscles to the effects of the water.

The cases for which Mr. Leichtenstein recommends his method are very different from the cases in which I used this method with such excellent results, and I was not as yet in a position to observe the clearing effect in the thinning of the leuchæmia human blood. But this by no means deters me from unreservedly recommending this method in all such cases of protracted turbidity as have been investigated by Mr. Leichtenstein, and, of course, cases of leuchæmia and leucocythæmia may present themselves to a practising physician.

There are indeed conditions so simple and so universal that the certainty which the word of a reliable observer gives cannot be increased or diminished by repeated assertions.

The testing of a definite blood solution is a task of so great precision that in the unanimous reports of all the different

universities conducting experiments, the various reports of one or more persons in the same blood test never varied more than one per cent.

The more deeply the blood solution to be tested is coloured, and the thicker, accordingly, that part of the glass wedge which is of the same colour, the more light the dull white reflector will throw through the comparing vessel.

If one is aware that the blood is normal, it is best to give the reflector such a position that as much light as possible will be thrown upon the lower surface of the vessel. But in such cases where the thinner parts of the wedge are brought into use, that position of the reflector must be sought which supplies a sufficient degree of brightness.

The universal results from the Hæmometer are, the sharper and more exact the smaller the degree of brightness used in obtaining them.

The observing eye must be brought at a certain distance, perpendicularly over the comparing vessel; the other eye must be closed. It is also recommended to place between the observing eye and the comparing vessel, tilted upon the latter, and standing upright upon the table slab of the Hæmometer, a cylinder of paper or pasteboard. The length of this cylinder must, of course, be suited to the sight of the observer. It will do no harm to have the inner surface of the cylinder painted black. The observance of the following rules is of the greatest importance :—

The observer should not place himself in a position toward the Hæmometer such as he would, for example, assume in the use of the microscope, but should place himself in the same plane with the partition wall of the comparing vessel. The consequence of this is that the picture of both, according to their colour and brightness, with comparative exactness semi-circles upon the retina, lie beside each other, not, as in other cases, one upon each other.

But the comparison of the degrees of brightness is much more exact when the impression is made upon the right and left halves of the retina, than upon the upper and lower halves. Such is the case for the following reasons :—

If one excludes the most peripheral portion of the retina in cases where there is a difference in the shape of the nose root on

the temple side of the retina. The right and left halves of the retina of an eye are generally during the whole life affected by light and shade to the same degree. In other words, they are blended in the same degree, and consequently are equally sensitive to light. The upper and lower halves of the retina, on the contrary, are subject to the effect of light in essentially different degrees, in that the picture of the firmament, which in general represents by far the brightest part of the range of vision, is always wanting in the lower half of the retina. Thereby it is kept more nearly blinded ; that is, less sensitive to light.

The observer must also take care that the observing eye is not affected by rays from the light which illuminates the Hæmometer. For in this case, in consequence of the lights penetrating the tissues (tunice) of the eye, a similar inequality between the two sides or retina halves may result, such as we have just found in the halves of the rétina lying one over the other.

The real work now is to focus the Hæmometer. This is done by moving the glass wedge by means of a large hand piece back of the column until the difference in the appearance of both halves of the comparing vessel has disappeared. This movement, as soon as the neighbourhood of the real graduating point is reached, should be backward, and by short, quick strokes, rather than by a constant slow motion.

The paths of the wedge as it is shoved from one side to the other over the proper point should be gradually shortened ; in this way the distance traversed is lessened while the decision vacillates, until one has at last decided upon the graduation.

As it is advisable to look often rather than long into the instrument, so also when the graduation point is supposed to be determined, the eye should be averted for a short time either by closing it or by looking at some dark surface, and then both halves of the vessel should be again compared. If there be the slightest doubt, the perfect equality of both halves should again be sought by short backward movements of the wedge, until at length further observation can detect no change in the decision either as to the purport or as to the exactness.

The sense of perfect exactness and unconditional correctness of the decision will be experienced in each case at the same time

with the conviction that the greatest care and attention has been given. In the use of the Hæmometer, which is so simple that it must be intelligible, the conscience of the observer will in every case tell him of how much confidence he has made himself and his observations worthy.

But when the observer has been able to reach only a hesitating and unsatisfactory decision, it cannot always be attributed to want of conscientious care and attention.

There are persons who, although they are not exactly red blind, nevertheless have a retina very sensitive to long undulations of light, and to such persons the graduation of the Hæmometer not only presents a certain difficulty while it does not allow them to reach a positive conclusion satisfactory to themselves, but according to the few experiments of which they were hitherto capable, it seems that such persons graduate the Hæmometer about one-fourth too low ; that is, in the examination of normal human blood at about 75 per cent.

Whether such persons can use the Hæmometer to advantage, and to what extent, and under what conditions, are questions to which the preceding experience can give no definite answer, and whose solution remains for future investigation. Still I wish to express, *a priori*, the following conjectures :—

In those who are severely suffering with red-blindness, whenever their retina are carefully studied and accurately observed, it is found that the same anomaly exists in all. Such cases afflicted with red-blindness manifest a functionary defect of the sense of colour.

I consider the validity of the same course of reduction-quotients for the totality of the red-blind even more probable than the validity of the same quotient for the whole extension of the Hæmometer-scale, every graduation made by one who is red-blind in any definite direction upon the Hæmometer-scale, always through this, one quotient should be changed into the corresponding graduation of the normal eye.

The inability to see red in its proper degree of intensity seems to be a functionary defect of the sense of colour, which occurs in all degrees between the normal eye and the total red-blindness. And I am not as yet convinced that in all the cases the defects

extend to and spread wave-like or in a constant ratio over those lying within the defect.

Under such circumstances it seems to me to be highly improbable for red-examiners to have such a common factor of reduction such as we have observed for the total red-blindness may exist.

In contradistinction to the above mentioned rare cases of eyes that are not at all able, or only to a certain degree able to use the Hæmometer, there are many observers whose sense of colour is in the beginning, or at least after a little practice, so keen that they are able to detect with the greatest exactness the inequality of the colouring in the part of the wedge suddenly made visible through the comparing vessel. Of course the difference in the thickness of the wedge at both ends of a piece in a position of the same visible at the same time is not less than 0.9 mm., therefore the difference in the graduation of normal human blood amounts to about 18 per cent. of the central thickness of the wedge. Yet it has been said that every observer is not capable of detecting the corresponding variation of the colour in the thickness of the red glass. Together with the ability to distinguish such slight differences in the intensity of the colour, there is combined a real advantage in the use of the Hæmometer. Such observers are able in graduating to seek that position of the wedge in which at the end of the partition wall of the comparing vessel the blood half is more deeply coloured than the wedge half; at the other end the wedge half appears darker than the blood half. Between these there must of course be a point at which the intensity of colour is the same on both sides of the partition wall, and this point must be in the centre of the partition wall if the increasing variations are alike at both ends. To carry out this arrangement the division of both halves of the coloured circle into three subdivisions (so that there are six in all) by means of two thin black straight lines perpendicular to the dividing line and dividing the latter into three equal parts, is advantageous.

I believe that I not only anticipate correctly the surprising effect which these directions for the use of the Hæmometer will probably have upon the most of my readers, but that I will also find this impression well founded by the evident incongruity

between the small number, the simple character, and the rapid execution of the proceedings demanded in Hæmometer measuring on the one hand, and on the other, great number of rules and instructions which I have given above. Since all should be alive to the importance of the cautionary rules for the correct execution of these proceedings, it cannot be otherwise than that every one will find in these instructions much that he already knows or considers self-evident, but it may also be that each will find something new or something which he himself would not have arrived at. The purpose in giving at length these rules is to enable each possessor of a Hæmometer to use it without fruitless attempts. In the very beginning he should make useful and reliable measurements. The purpose could be fully carried out only by a complete enumeration of all possible rules that might be considered.

Chemistry and Palæontology.

A NOVEL application of Chemical Analysis was recently explained by A. Carnot at the Paris Academy of Sciences.

He has endeavoured to fix the age of prehistoric human remains by noting the progressive diminution of fluorine in the fossil bones of successive geological ages. Representing the proportion found the oldest specimens as 1, that of the tertiary remains would be indicated by 0·64, of the quaternary by 0·35, and of the more recent bones by 0·05 or 0·06. An opportunity of testing the value of these figures was afforded by the discovery of a human tibia at Billancourt (Seine), near some animal remains of the quaternary period. There was a difference of opinion as to whether it was of the same period as the other fragments or not, but since on analysis the proportion of fluorine in the animal bones was found to vary from 0·469 to 0·578, as compared with 0·066 in the human tibia, the more recent origin of the latter was regarded as established.

—*Comptes Rendus.*

A Device to take the place of the Camera Lucida in Micrography.

BY HENRY G. PIFFARD, M.D.

THE act of micrography, or the reproduction on paper of images of minute objects seen through the microscope, may be practised in various ways, of which the three following are the principal :—

1.—The observer studies the object on the slide, and, when he thinks he has the outlines and details, or a portion of them, sufficiently impressed on his mind, withdraws his eyes from the tube, and commits the mental picture to paper, using, of course, both eyes in directing the movements of his pencil. Success with this presupposes a retentive memory and considerable skill as a draughtsman.

2.—The observer, looking down the tube in the usual way with one eye—for convenience, the left—is, after a little practice, enabled, by a sort of auto-projection, to see an image of the object on a sheet of paper by the side of the microscope. The outlines of this image he traces with the pencil, using the right eye to direct its movements, the observation and the reproduction being simultaneous.

3.—By the aid of a camera lucida, of which there are many different sorts, a reflected or projected image is visible on the paper with the eye that is at the same time occupied in directly observing the magnified image of the object on the stage. In one of the latest forms of camera lucida—the Abbe—this use of half the eye for observing, and the other half for recording, is a reasonably convenient method, if the observer's eye is approximately normal ; marked myopia or hypermetropia, and still more pronounced astig-

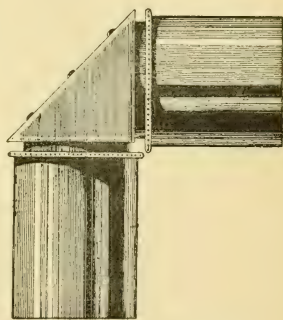


FIG. 4.—The author's drawing prism.

matism, necessitating the use of spectacles, render the use of the camera lucida inconvenient, if not well-nigh impossible.

Some time since it occurred to the writer that the practice of micrography could be greatly simplified by adapting the principles employed in ordinary projection, as used in connection with the optical lantern, the projection microscope, photo-micrography, etc. It was only a question of reflecting the projected image on to a piece of drawing-paper fixed in some convenient position. To this end I requested Messrs. Bausch and Lomb to mount a right-angled, reflecting prism with a short tube extending from one of its square faces, (Fig. 4), this tube to be of such calibre that it could be inserted into the microscope in the place of the eye-piece. From the other square face a similar short tube extends, capable of receiving the ocular and holding it firmly.

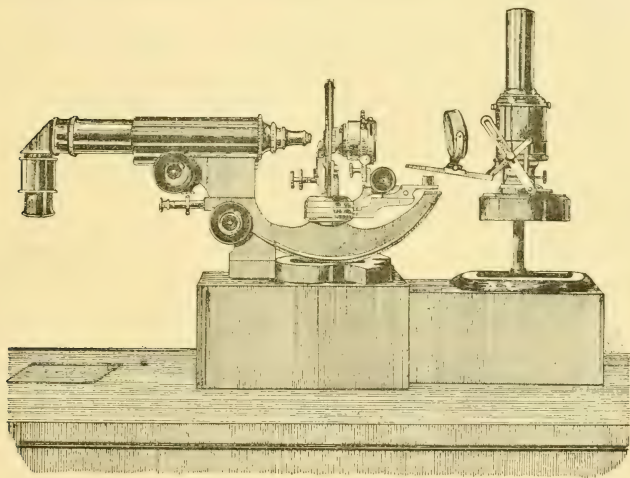


FIG. 5.—Showing lamp, microscope, and prism in position.

When preparing to use this device the object is placed on the stage, and focussed in the usual manner. The microscope is then brought to a horizontal position, the eye-piece is removed, and the prism case put in its place, the ocular being inserted in the short tube provided for its reception. The ocular should point downward. The lamp, or other source of light, should then be disposed in such a way that it properly illuminates the object to be examined,

it being expressly understood that no light shall escape toward the observer, except that which first reaches the object. A Beck lamp is conveniently adapted to this purpose. If a piece of drawing-paper is placed beneath the ocular, and the room darkened, a brilliant image will be projected on the paper, and its reproduction can be easily accomplished with a maximum of rapidity and a minimum of discomfort. In guiding the pencil the draughtsman uses both eyes, and his spectacles if needed, and sits in whatever position he finds most comfortable.

The general disposition and arrangement of the apparatus will be readily understood by an examination of the accompanying cut (Fig. 5).

With a proper lamp, and careful utilisation of its light, this device gives excellent results with amplifications up to four and five hundred diameters.

If a sensitive photographic plate be substituted for the drawing-paper, an exposure of a few seconds will impress an image that may be developed in the usual way.—*New York Medical Journal*.

Mosquitoes are said in the *Quarterly Review* to have been frozen on to the surface of a lake in the evening, and thawed again by the morning sun into animation. Alpine climbers sometimes pick up butterflies lying frozen and brittle on the snow, which revive and fly away when taken to lower warmer regions. Insects which habitually hibernate, as larvæ and pupæ, do not suffer from being frozen for a lengthened time; but they suffer in open winters from frequent alternations of wet, warmth, and cold.

THE ASTEROIDS.—According to calculations by M. L. Niesten, all the asteroids known (now more than 300), if combined into one, would form a body not quite 514 miles in diameter, or less than one-twentieth the diameter of the earth; and it would require 8,575 bodies like it to form a planet having the volume of the earth. The largest of the asteroids, Vesta is 230 miles in diameter, and the smallest Agatha, four and-a-half miles. As all these bodies having considerable size have most probably been discovered, the estimate of the mass of the whole is not likely to be materially affected by the detection of new ones.

Preparing Sections of Teeth for Histology and Bacteriology.

PART II.—HISTOLOGY PRACTICAL.

BY PROF. V. A. LATHAM, D.D.S., F.R.M.S., ETC.

Section II.—SOFTENED SECTIONS.

FOR this purpose several reagents may be used, but all seem to act by the removing of the calcareous matter and the hardening of the soft tissues of the teeth. It is most important that the last should be thoroughly well attended to, or else we do not get a correct idea of the character and structural size of the elements found in the tooth. Chromic acid is the first; a one-sixth per cent. up to one-half may be used. The former is preferable, and is made by adding one gramme to 600 cc. of distilled water, or 15 grains to the pint, a quantity of which should be kept on hand in order to immerse a freshly extracted tooth that may be desired for examination.

Tie a piece of cotton round a fresh tooth, and suspend it in the fluid (one part of spirit to two parts acid), carefully stirred so as to be covered by a quantity of the solution. Where the crown of the tooth is not required for examination, it is better to saw it off at the neck so as to allow the stains and reagents to penetrate the tissues. The hardening fluid must be changed the second day and on the fifth and eighth days. On the ninth day it is placed in a mixture consisting of two parts of spirit and one of water well stirred; and on the tenth day into pure spirit and left till desired to use. If desired to soften at the end of the eighth day, a few drops of hydrochloric acid can be added to decalcify, which may be tested by passing a fine needle through the tooth; then wash well to remove the acid; then place for a day into two parts spirit and one water, and the next day into ordinary spirit; on the fourth day embed and cut.

To Embed in Paraffin.—A small tin or paper mould should be made and some melted paraffin poured into it. The tooth is then quickly dipped in and withdrawn till quite cool. As the paraffin is now cooling, place the tooth in it near the hand of the

operator, and when set withdraw the needle. Place it in a cool place to harden. To prevent the object becoming displaced, it should first be dipped in alcohol for some minutes in order to dry the surface thoroughly. The sections are then cut with a razor and kept flooded with alcohol, and the sections floated off the knife into a capsule of the same. If desired, a mixture (by weight) of white wax and olive oil, equal parts, melted together and poured into the embedding dishes, may be used in place of the paraffin.

To Embed in Gum.—If the tooth is in alcohol, transfer for six hours into distilled water, then to a gum solution for six hours (made of picked gum arabic dissolved in water). Place a fair quantity of the gum on the plate of the microtome (Cathcart's or Williams'), and cool till nearly frozen. Place the tooth in position and surround with gum, and let it freeze under a capsule, or by means of the spray, till solid, but not brittle. The gum should cut like cheese. When the sections are cut, transfer with a camel-hair pencil from the knife to warm distilled water to dissolve out the gum. From water transfer to spirit and spread the sections out evenly. If the sections are delicate, they may be transferred directly to the slide and treated with dilute spirit *insitu*. These may be washed with water and stained with any agent or gold chloride, and subsequently dehydrated and cleared and mounted in any media.

Decalcified Sections.—Picric Acid.—A saturated solution in distilled water is the safest decalcifying agent, and it must be kept saturated by the addition of fresh crystals every few days. When soft enough, prick with a needle to test it, and then wash well in clean water to get rid of the acid and then in weak spirit, which will dissolve more acid. Keep in pure alcohol and treat as chromic acid for embedding, cutting, etc. Sections may be double stained with picro-carmin and logwood, etc.

Muller's Fluid.—This is one of the most useful hardening agents we possess ; it consists of bichromate of potash, 2 parts ; sulphate of soda, 1 part ; and water, 100 parts. Put the salts into a pan with some of the water and boil till all is dissolved. Add the rest of the water to cool it, and put in a stoppered bottle to keep. After the first few days it does not require changing, but requires longer time to harden according to the size of the tissue,

and is a great advantage, as specimens are in no way harmed for the demonstration of micro-organisms. When the tissue is properly hardened, pour off the hardening agent and wash well with water to get rid of the reagent, transfer to weak spirit for a day or two (2 parts spirit, 1 water, stir), then into pure spirit.

Sections of Pulp.—Crush a newly extracted tooth in a vice or with a hammer, and select several pieces of dentine with portions of pulp adherent to them, then immerse in staining fluid, cover with a glass capsule, and leave in a warm place for a couple of days. Then pour the fluid off and wash the specimens in a solution made of strong glycerine 2 parts, distilled water 1 part, and leave for a couple of hours to soak. To enable the tissues to regain their original volume, transfer them to a solution of 5 drops acetic acid to 1 ounce of strong glycerine, and leave in the fluid for four days.

Another method and one recommended by Dr. Bodecker for preparing pulp tissue is to immediately place the tooth after removal from the mouth in a one-sixth to one-half per cent. chromic acid. To this mixture add, after the third or fourth day after decalcification, a few drops of dilute hydrochloric acid. It is important to use a large quantity of the liquid and renew every six or eight days. After the teeth have been hardening and decalcifying for a few weeks, the peripheral portion of the dentine will become sufficiently soft to be cut with a razor. When the hard portions of the dentine are reached by the cutting instruments, the extraction of the lime-salts must again be continued in the manner described above until the pulp cavity is reached. If care is used, the tooth, especially the anterior, can be split with a strong pair of excising forceps. Then take a half per cent. solution of sodium chloride in distilled water (warm), and place on the pulp and drop it without handling into the stain, carmine, logwood, fuchsin, hyperosmic acid, picro-indigo, or chloride of gold, half per cent., etc.

Pulp sections, if thin, after hardening in chromic acid, can, if first washed in distilled water, be stained with gold chloride, leaving the stain on the tissue for twenty to thirty minutes, when they should again be washed in distilled water and exposed to daylight. In a few hours the colour of the fresh tissue changes to a bright violet colour, whilst the chromic acid pulp is of a brownish violet.

Osmic acid, 1 per cent., shows the contours of the constituent tissues, the nerve-fibres being more especially distinct. Again, both fresh and chromic acid specimens may be treated with osmic acid. Carmine is, perhaps, the best stain for pulps.

To examine the pulp together with the enclosing dentine, the specimen is softened in chromic acid and then embedded in celloidin, or paraffin, or wax, as above described. The fresh pulps of lower incisors are the thinnest and best adapted for examining the system of blood vessels, and if transferred to the slide when fresh, add some normal saline solution, cover, and with careful pressure the pulps may be spread and examined.

The Blood-Vessels of the Pulp, To Study.—Chloroform an animal, and just before respiration ceases open the right auricle and let the vessels empty themselves; then inject with Prussian blue, warmed to a temperature of 40° C. to render the gelatine fluid, and also to prevent any vascular spasm which a cold fluid is very liable to produce. Then place the head in alcohol for twenty-four hours to harden the injection; or, if preferred, in Muller's fluid or chromic acid, which is quite as good and in my opinion better. The pulp, after hardening in alcohol, is removed and immersed in a weak solution of chromic acid, and at the end of ten days sections of it may readily be cut and then mounted in glycerine jelly. If the animal is quite dead, you must wait till *rigor mortis* has passed off and inject a non-gelatinous Prussian blue, but the first injection is the best. In animals which die of strangulation the vessels will be found so gorged with blood as to render any further preparation unnecessary.

If the tissues are partially decalcified in a very weak solution of chromic acid and treated as above described, sections can be frozen, cut, stained, and mounted, so as to show the dentinal fibrillæ as prolongations of the odontoblasts.

I prefer to harden teeth well in Muller's fluid, then spirit, and then to grind sections, keeping *them wet* all the time, and if wished they can, after grinding, be embedded in celloidin and decalcified in half per cent. solution of chromic acid, then treated and stained as desired. A point worthy of remembrance is the dissimilarity between caries of bone and decay of teeth, as the reaction is totally different when they are treated with picro-carmine.

Perenyi's Fluid.—As yet I have had very little chance to thoroughly test this agent, to which Dr. N. S. Hoff, of the University of Michigan, very kindly drew my attention, but it promises to be a good agent, having the properties of hardening and decalcifying at the same time. The formula is :—Nitric acid (10 per cent.), 4 parts ; alcohol, 3 parts ; and chromic acid (0·5 per cent.), 3 parts. Mix. Leave the tooth in this agent some four hours or more, until soft. The softening is facilitated if the tooth can be cut through at the neck so that the agent can enter the canal. Then transfer to 70 per cent. alcohol for twenty-four hours, strong alcohol for some days, absolute alcohol four or five days. For ordinary tissues combine the stain with the fixing fluid. Fuchsin may be dissolved directly in the fixing solution. But eosin, purpurin, or aniline violet must first be dissolved in three parts of alcohol, and then shaken into the liquid.

Picro-carmin and borax-carmin can also be added, but as a precipitate results it must be filtered, and after staining pass through 50 per cent. alcohol for five hours ; ordinary spirit, ten hours, and then into absolute alcohol. If the tissue is unstained, it may, after cutting, be immersed in clove oil coloured with an alcoholic solution of eosin or safranin, or it may be placed on the slide for five to ten minutes, with a few drops of the coloured oil.

Kleinenberg's fluid is also useful for decalcifying teeth.

To Clean Cover-Glasses.—Take the cover-glasses from the wool in which they are usually kept, and place them in a beaker, cover with strong sulphuric acid ; stir, to get rid of all the air remaining amongst them and leave them for an hour or two. Then wash well in *several* changes of water to get rid of the acid, and place in alcohol, from which they are taken one by one, and wiped dry on an old handkerchief, and then polished with a chamois leather and kept for use in an air-tight box.

Retaining the Soft Parts of Bone and Teeth.—Take a fresh, or nearly fresh, tooth. Divide it *at once* with a sharp fretsaw below the neck into two or three pieces, “allowing distilled water to trickle over it the while,” and then the reagents and stains can penetrate the pulp cavity. Place the pieces in concentrated sublimate solution for some hours to fix the soft parts. Wash in running water for about one hour ; then place for twelve hours in

30 per cent. spirit, change to 50 per cent., and again after a similar period to 70 per cent. To remove the black sublimate precipitate, place the teeth for twelve hours in 90 per cent. spirit, to which 1.5 to 2 per cent. tincture of iodine has been added. The iodine is afterwards removed by immersion in absolute alcohol until the teeth become white.

To Stain in Borax Carmine, either an alcoholic or aqueous solution gives the best results. Remove the teeth from absolute alcohol to running water for fifteen to thirty minutes, and then place in the stain. In the watery solution of borax carmine they must remain one or two days, and in the spirituous two or three days. Transfer to acidulated spirit (70 per cent. spirit, 100 parts; muriatic acid, 1 part), in which they may remain; the watery ones stained require at least twelve, and the alcohol stained ones twenty-four to thirty-six hours. This done, immerse for about fifteen minutes in 90 per cent. spirit and then for half-an-hour in absolute alcohol; after which they are to be transferred to some ethereal oil for twelve or more hours; then quickly wash the ethereal oil off the objects with pure xylol. They are then to be passed into a solution of balsam in chloroform.

The Balsam is prepared by drying in a water-bath heated gradually up to 90 deg. for eight hours or more until, when cold, the mass will crack like glass on being punctured. Of this balsam so much is added to the chloroform as to make a thin solution, in which, as stated above, the teeth must lie for twenty-four hours. Then add as much balsam as the solution will dissolve. When no more balsam will dissolve, the teeth and a sufficiency of the balsam are poured into a vessel and heated up to 90 deg. in a water-bath until the mass, when cold, shall be as hard as glass. Let the balsam set; pick out the teeth carefully, place in a vice, and thin discs are cut from them with a fret-saw, water being trickled over them the while. Mount sections in chloroform balsam.

Teeth to show Odontoblasts in situ.—Take the jaw, preferably the lower jaw, of an embryonic mammal such as a kitten or puppy, and while still fresh carefully strip off the tissues covering it, except the oral epithelium and flange of gum, and place in the usual Muller solution, twenty to thirty times in volume greater than the

bulk of the immersed tissue. Change the fluid every day for four or five days, and then every third or fourth day. Then finish hardening after it has been in Muller's fluid for a fortnight, first in weak spirit, then into strong, changing till no more colour is seen. Vertical sections are cut by a thin, sharp knife; place longitudinally on the stage of a Cathcart or Williams' freezing microtome and cut in the usual way. The best specimens are got in the canine and bicuspid regions, for these parts are less likely to be disturbed in the manipulation processes. Embedding in wax, paraffin, or celloidin is of little service. The knife cuts the thin cap of semi-calcified dentine and bone quite easily, and the elements of the pulp are in no way disturbed in their relation to each other. The odontoblasts can be separated, if necessary, by separating with the point of a needle from the surface of the dentine papilla, the cap of dentine to which, in places, they adhere. This affects little, if at all, the relative positions of dentine, odontoblasts, and pulp.

To make Preparations of the Teeth of Fishes in situ.—It is best not to grind down sections of the teeth, but to decalcify the jaw and teeth with a 5 per cent. solution of hydrochromic acid or a 10 per cent. solution of hydrochloric acid. Cut sections, stain and wash them well in distilled water, dehydrate for three minutes in absolute alcohol, clear in clove oil or xanthol, and mount in Canada balsam. Carmine is, perhaps, the best stain for fishes' teeth. If it is used, however, it is necessary, before transferring to distilled water, to pass the sections quickly through weak acetic acid, as this fixes the stain. If gold chloride is used, the specimens must be mounted in glycerine jelly.

It is not necessary to Cut Sections of Enamel to Demonstrate the Prisms.—1st, Soften the enamel by immersion in a 10 per cent. solution of hydrochloric acid. By means of a needle point or fine brush, remove a small portion to a slide; put a drop of normal salt solution on the top of the enamel and press down the cover-glass; then run a solution of carmine or orange-rubine beneath the cover-glass, and draw off the excess with a small piece of blotting-paper. Wash the stain away further by irrigation with a weak hydrochloric acid or acetic acid solution, and mount in this solution or acidified glycerine after Beale's plan.

Staining with Chloride of Gold.—No other stain marks out so clearly the minute anatomy of the soft tissues which penetrate bone and dentine ; in fact, its excellence as a selective stain would long ago have been demonstrated but for the recognised text-books speaking of the great difficulty of using it and the length of time it takes, and being only applicable to fresh tissues.

True, fresh tissues always stain faster ; but teeth and bone, and indeed other tissues, can be stained after having been severed from the living body for a long time—sometimes weeks. Avoid the use of metal instruments, bone, wood, or quill being preferable. The use of steel does not, however, doom the staining to failure. To stain (*a*) wash the sections in a solution of bicarbonate of soda. (*b*) Put some 1 per cent. solution of gold chloride in a watch-glass, test it with litmus paper, and, if acid, neutralise with (*c*) bicarbonate of soda by drops. Place the sections in the solution and cover the watch-glass with a lid to keep it in total darkness for from half to one hour until the sections are straw-colour.

Remove the sections from the staining fluid to distilled water, leave covered over for a few minutes (they must not be exposed to the light for more than a few seconds). (*d*) Put some 1 per cent. formic acid in a watch-glass, float the glass on hot water, put the sections in the acid, cover them over and keep them in the dark and fairly hot until they turn crimson, which will be in about an hour. (*e*) When stained, immerse the sections in cold distilled water for about half-an-hour. (*f*) Dry the sections and mount them in glycerine jelly; avoid Canada balsam. Keep gold chloride bottle in the dark.

Sections to show pulp (particularly hyperæmia) are difficult to make and to retain the natural injection. First, Catch your hare—*i.e.*, capture the condition—examine a suitable case when one is presented, note condition of the tooth itself, etc. Remember *the condition of the tooth at the moment of extraction, especially as to pain*, as it is of vast importance in studying this object. Extract this tooth ; place in Muller ; do not handle or disturb in any way for a week at least, Then harden ; wrap the tooth in muslin and place in the jaws of a powerful vice (not one where the jaws are so weak that they will spring together on cracking the tooth, as it will crush the pulp), and steadily close them until the tooth cracks

open. If skilfully done, the line of fracture will be the long axis. Then place in Muller's fluid, freshly filtered, and carefully lift the pulp from its cavity. (Carefully do this, for the dentinal fibrils will be pulled out a considerable length.) Now place in a thin solution of gum arabic, to which should be added some gum camphor, salicylic, thymol, or carbolic acid, to prevent mould. *In no case must this gum be strong enough to float the pulp.* If of greater specific gravity than the pulp the tissue shrinks. Evaporate the gum arabic solution slowly to the consistence of a thick jelly. This should require three or four days to thoroughly penetrate the pulp. When the solution is hard enough to handle, the pulp is taken up with some mucilage, placed in the position for cutting on a piece of cork afloat on alcohol, with the pulp side down. In from twelve to thirty-six hours the surface will be considerably hardened by the abstraction of the water by alcohol. Do not let it get too hard. Insert in a microtome; use paraffin or some suitable substance for embedding, and allow to stand twelve to twenty-four hours. Several sections can be cut if desired, the specimen being kept wet in alcohol all the time. Mount direct in glycerine without dissolving the mucilage, or dissolve it out in tepid distilled water, stain the pulp with hæmatoxylin or fuchsin, and mount in Canada balsam.

If a tooth is extracted during a paroxysm of pain, inflammation of the pulp is almost uniformly accompanied by the signs of hyperæmia, they being present in a marked degree in the immediate neighbourhood of the inflammatory area; but if the tooth is extracted during a period of quiet, the hyperæmia is limited to the vessels within the inflamed area.

Celloidin.—After well hardening, place for twenty-four hours in equal parts of ether and alcohol, transferring to a syrupy solution of celloidin, made by dissolving celloidin in a mixture of equal parts of alcohol and ether. Leave it for about twenty-four hours; cover the object with a thicker solution of celloidin, and allow it to remain in it for twenty-four hours. When ready, embed on cork. Spread on the cork a little of the celloidin solution and allow to dry; then another coat and let dry. Now place on it the specimen as quickly as possible before the celloidin begins to harden. Then cover the whole with successive layers of the celloidin solution

until the object is built up quite firm. When it has dried remove the celloidin from the glass with a sharp knife, and if necessary trim the mass to a proper size and form.

To place on Cork.—Coat the cork with celloidin solution and let it dry (to prevent air rising from the cork). The object is now placed in its hardened matrix and mixing cell, as on the cork, by means of celloidin. Let dry in air till it retains its shape well. Drop the cork into 50 per cent. alcohol, and it can usually be cut after soaking it for one hour. For dental embryological work it is excellent.

Developing Teeth Sections.—Take the teeth that are forming in the jaws of embryos, at or nearly the time of birth, while the tissue is still warm if possible. Place in $\frac{1}{4}$ to $\frac{1}{2}$ of 1 per cent. solution of chromic acid and change daily for three or four days. The edges of the dentine that were calcified are found sufficiently softened to make a number of sections. Take the teeth from the acid solution, wash in distilled water, and then place in a solution of gum arabic for several hours. Then put in alcohol to take out the water. Paraffin and wax are melted and poured into a convenient mould. When clouded with cooling, embed the tissue, and cut it until the calcified tissue is reached. Place the sections in distilled water for a few minutes to dissolve out the gum, and then put in glycerine and alcohol and mount in glycerine.

For further details on Embryonic Teeth Sections, see paper on Histology of the Teeth in this Journal, New Series, Vol. II., 1889, where they are given at length.

Preparing Sections of Decayed Dentine.—Select a freshly-extracted decayed tooth, wash out all the particles of food, and break away the margins of enamel so as to expose the softened dentine as much as possible. Then with a sharp instrument cut away the decayed portion from the sound dentine, keeping the instrument *well* to the latter, and we thus get a large piece of decayed dentine. Immediately freeze the tissue in gum, stain and mount.

Staining Tissues.—This requires a little practice to secure good results, so we will take the simplest—namely, logwood. Buy a good sample, or make one from the many receipts found in all

histological text-books, and then filter twelve or fifteen drops in a watch-glass, and add a few drops of distilled water; stir well to mix the agents. Place in a few sections (the fewer and the slower they stain the better) from the spirit, and let them straighten out on the stain, and then gently press under the logwood; leave it for about ten minutes (the time differs in every case), and then test them by washing in *tap water*. This is about the only time we have occasion to use any other than distilled water, but the former fixes the stain the best. When deep enough and well washed to remove all precipitates, place the sections in spirit for a good ten minutes to dehydrate, then in clove oil, and finally mount in Canada balsam, or what other media is desired. All the stains are used, with only slight modifications, in a similar manner.

Picro-Carmine is a very useful stain on account of its double-staining property. Place the sections in a strong solution for from ten to thirty minutes, then wash in acidulated water (distilled water to which one or two drops of acetic or picric acid have been added). Leave in this for fifteen to thirty minutes, wash quickly in alcohol, and then transfer to clove oil and mount in balsam. Some histologists are against the use of balsam as a medium, and advise glycerine or Farrant's media. Logwood is a good combining stain with the above. Fuchsin is also a good stain.

THE MEXICAN jumping seed, or "Devil's bean," is a euphorbiaceous plant of such poisonous properties, that it is used by the Indians to envenom their arrow-points. It not having been scientifically identified to satisfaction, Dr. C. V. Riley has made a special study of it. The saltatory property is not intrinsic with it, but is imparted to it by an insect (*Carpocapra saltitans*), which secures lodgment within the bean and does the work. Dr. Riley believes that the insect is developed in the capsules of several species of the genus *Sebastiana*.

On the Cultivation of Diatoms by Artificial Means.

BY DR. MIGUEL.

Translated from *Le Diatomiste*.

CHAPTER I.—THE ORDINARY GROWTH OF DIATOMS.

TO cultivate any microscopic species it is necessary that the conditions of its independent and voluntary reproduction should be provided by the experimenter.

Such an operation requires—

1.—The formation of a nutritive medium, suitable for promoting the development of the species.

2.—The subsequent sowing of the microphyte, the multiplication of which is desired.

3.—Special precautions, without which the growth would be endangered, and which consist essentially in promoting the life of the microphyte, either in avoiding the many things which would injure it, or which would increase too greatly the action of such as stimulate it.

1.—*The Cultivation of Diatoms in a fresh-water Preparation of the Nutritive medium.* Besides the natural medium for the growth of diatoms, which is water, it is necessary to provide them with two kinds of food : Saline food and Organic food.

The common water of springs and streams are not usually sufficiently charged with mineral and organic substances to favour an abundant growth of diatoms ; nevertheless in sowing diatoms in common water, fully exposed to light, you can see produced at the bottom of the vessel, especially if the vessel be of glass, little yellow spots, formed exclusively of diatoms in course of multiplication. But these organisms soon exhaust the mineral and organic constituents which are essential to their support, and its growth is arrested. It is resumed and carried forward as soon as these constituents are supplied ; how this is done we are about to show.

It is only after having studied repeatedly the action of different salts that I have been able to establish a proper formula for

mineralising water, the addition of which will give good results in the culture of siliceous algæ in fresh water. The salts of soda and of lime are those for which diatoms have a special liking, and it is the same, though in a less degree, with the salts of potash, while those of ammonia are often injurious.

To mineralise a liquid suitably you may add, with good effect, to a litre of common water, 40 drops of solution A and from 10 to 20 drops of solution B :—

A.

Sulphate of magnesia	10 grs.
Chloride of sodium	10 "
Sulphate of soda	5 "
Nitrate of ammonia	1 "
Nitrate of potassa	2 "
Nitrate of soda	2 "
Bromide of potassium	0.2 "
Iodide of potassium	0.1 "
Water	100 "

B.

Phosphate of soda	4 grs.
Chloride of calcium, dry	4 "
Acid, hydrochloric, pure, 22°	2 cms.
Perchloride of iron, liquid, 45°	2 cms.
Water	8 cms.

Thus if the volume of water is only 50 cms. you must add 20 times less of these liquids, about 2 and 1 drops of each solution.

On a pinch you may dispense with the mineralisation of the ordinary water, and the reason for this is based on the fact that vegetables, which give food to diatoms, contain for the most part the above-mentioned elements in a state of combination, but experience shows that the decomposition of these vegetables being always very slow, the diatoms have generally an excess of organic material and not enough mineral, which retards their development. Observers may attempt the growth of diatoms in common water, and may be sure of obtaining growths, often very beautiful, but rather sparse. Besides, they must not forget that the formulæ I am giving are capable of great improvement, and that they may

be greatly modified, according as it is desired to hasten the multiplication of such and such diatoms.

Some fresh-water diatoms require for their maximum development increased proportions of the salts. I have known some that will very well take 40 grains of chloride of sodium to the litre—others 10 to 15 grains of nitrate of soda—many develop strongly under the action of powerful disinfectants. I cannot, in the short exposition that I propose to make here, enter into these different questions, and on these points I would refer the reader to articles that have already appeared, on the Physiology of Diatoms, in the *Annale de Micrographie*.

The ascertaining what organic substances are suitable for the culture of diatoms has required a great number of experiments—thus in my early experiments I tried leaves, twigs, and roots of a great variety of plants, and vegetable and animal tissues of every class; and it was by noting at each experiment the results as more or less favourable that I was compelled to eliminate the greater part of these substances, as sugars, gums, starches, the albumen of eggs and of blood, green plants, etc., and to retain only a small group of organic matters—as the bran of wheat, rye, and oats, the stalks of grasses, mosses, the dried excrement of the *rodentia* and *herbivora*, flesh (muscle), washed and cooked, and this last I was afterwards obliged to reject as it favoured the development of fungi and green algæ.

Finally, it is necessary to supply to the diatoms such substances as putrefy slowly and with difficulty, and I may add in quantity so small that the water in which they are immersed shall never at any moment, and especially at the beginning, show that phenomenon of active putrefaction which is indicated by the cloudiness of the mixture under the influence of bacteria.

A formula for culture which always gives good results is as follows:—Water 1,000, wheat bran 30 to 40 grains, with the addition of 1 decigramme of wheat straw and as much of moss; its nutritive power will be greatly increased by being mineralised in the way we have already indicated.

For cultures of less bulk than 300 cms. it is better to use powder bottles—that is, those with wide mouths which can be closed tightly with wadding to preserve the liquid from dust, insects, etc.

Cultures of greater bulk are more conveniently managed in precipitating glasses or in large glass vessels; the access of air to the surface of the liquid is necessary, not to furnish the nourishing elements to the diatoms, but chiefly to promote the diffusion of certain poisonous gases, notably, sulphuretted hydrogen, which is always produced in these cultural operations, and which the oxygen of the air transforms partly into water, sulphur, and sulphuric acid.

Having said so much, each experimenter can vary at will and perfect the formula I have given. My only object has been to publish, here, some general directions, by which it shall be possible, at once, to grow diatoms successfully.

As diatoms, like the majority of vegetables, take in nutriment by endosmose, and part with the residuary secretion by exosmose, the observer may, if he choose, dispense with the nutritive solids of which I have spoken; then he should prepare the macerations separately, and ultimately should sow the diatoms in the clear and limpid liquid resulting from an infusion made without heat, and continued for two or three weeks. But those media that spoil rapidly are the best adapted for the culture of diatoms in a state of absolute purity, in which case they must be filtered and placed in vessels, sterilised by being exposed to a temperature of from 110° to 150° . For full details of the preparation of these nutrient liquids, without the aid of heat, consult my recorded investigations. Finally, as the macerations in which diatoms can be grown are also suitable, in degree, to the multiplication of chlorophytes—especially *Desmidiaceæ*—it is well to avoid as much as possible sowing these green algæ.

2.—*Sowing the kinds of Diatoms.* This operation, which in ordinary cases is very simple, consists in introducing into the macerations some of the diatoms that you desire to cultivate. It is essential to point out here that the liquid must be sterilised as regards the green algæ, and such diatoms as may possibly be existing in the liquid after its preparation.

You can, with certainty, sterilise these liquids by placing them in a water-bath, kept for about half-an-hour at 70°C. , taking care to ascertain that all parts of the liquid are raised to this temperature. In practice it is convenient to use a tin or zinc vessel half

full of warm water, in which the flasks to be sterilised are plunged to a point above the surface of the contained liquid.

In the ordinary culture I recommend 70°C . as always sufficient, because diatoms perish at temperatures above 45°C ., and not to boil the liquid for two reasons; first, to avoid loading the liquid with too great a quantity of organic matter unfavourably modified by heat; and secondly, to avoid the precipitation of lime, which is soluble in water as a bi-carbonate, and which boiling changes to a neutral carbonate, almost insoluble in water.

The diatoms that you wish to multiply should never be sown in a state of dryness, for a desiccation of only a few minutes is enough to kill irrevocably those living frustules that are most charged with endochrome; possibly the spores of diatoms, if such exist, possess, like those of mosses and bacteria, the power of surviving a long term of drought, but this has yet to be established. In consequence, it is necessary always to introduce into these macerations, either diatoms held in suspension in water or the same algæ contained in moist receptacles, or such as are obtained by allowing the water to run away very slowly.

As to the sowing—that is, the introduction of the diatoms into the nutrient liquid—you can use the point of a pipette, previously made hot, or a wire of platinum, having its end flattened so as to act as a small spoon. The pipette, with a point finely drawn out, will meet most of your wants.

If you need to sow a single living frustule, the operation becomes much more delicate. It requires then the application of special, well-known methods—of fractional sowings—of Hansen's process—and of other methods that I shall describe later on in the second chapter of this memoir, devoted to the cultivation of diatoms in a state of purity, in which I shall show that you may simplify this operation by a first sorting of the diatoms by heat—by antiseptics—by nutritive media greatly modified—in a word, by all that tends to give a preponderance to the species you desire to isolate.

3.—*Of the management of the Culture.* The sowing being made the maceration should be exposed to the north, either in the open air or in the house behind a glazed window. Diatoms will not develop in the dark, nor in a half light. When the

actinic rays are deficient, these algæ cannot grow, but they preserve for a period of about four months the power of reproduction, when brought to the light of day. During the cold of the last winter I did not observe the development of any of the diatoms of our climate. From about 0° to 5° C. their rate of multiplication is very small; it is very appreciable between 5° and 10° C., and from that to 30° C. the temperature is favourable to the greater part of the species. Thus, to have a rapid and prosperous cultivation it is essential that the diatoms want neither heat nor light. These facts, and others well-known to all diatomists, receive here a simple confirmation from direct experiment.

The eminent diatomist of Belgium, M. le Dr. H. Van Heurck, who has had in his laboratory a spontaneous growth of marine diatoms ever since 1886, and which exists to this day, has remarked that the blue rays are favourable to the life of the diatoms; his observations are quite true—indeed, two classes of rays are useful to diatoms, the blue and the yellow; in the red rays the multiplication is insensible. Nevertheless, in my opinion, especially at the commencement of the growth it is well to use coloured glasses, which always produce some obscurity, and which by that means lessen the multiplication of the first frustules that have been sown. All interposition of glass, coloured or uncoloured, of vessels arranged to produce monochromatic light, produce numerous reflections, and offer a very serious loss of the actinic rays, which induces a delay and a slow pace in the multiplication of the algæ. Having said so much, the experimenters can easily, as I have myself done, expose the diatoms to white light which has passed through opalised, ground, or fluted glass.

I have been able also to prove that during the shortest days of the year, in the month of December, you can easily cultivate diatoms, if the place where the preparations are kept is not too cold, in which case a fire should be maintained for several hours, or, better, form a warmed glass case in the window, which could easily be done.

Under no excuse should the maceration be exposed to direct sunlight, for in our climate, after the month of March, this is sufficiently hot to raise the temperature at times to 45° C., a degree of heat fatal to diatoms. Under the action of the sun's rays the

golden-yellow spots of the best and most flourishing growths turn green, then lose all their colour, and at the end of the day the result is disastrous, all the diatoms have perished, and the liquid in which they are placed gives out an aromatic odour of aniseed, very like that of the bug. You may notice that in exposing to the action of the sun-light, two vessels, one containing a growth of diatoms and the other distilled water, that the temperature of the liquid containing the organisms may rise to 48° and 50° , whilst in that of the pure water, the thermometer only rises to 45° or 47° C.

The sowing being accomplished, you will see in from 3 to 10 days, according to the species sown and the existing conditions, yellow spots at the bottom of the vessel, and (if it be cylindrical, specially at these points, produced by the caustics of refraction), these grow rapidly from day to day. Soon the deposit occupies not only the bottom of the vessel, but also its upright sides; while bubbles of oxygen gas, released by the diatoms, rising incessantly, carry upwards some of the newly-formed organisms, and produce a mass on the surface of the liquid. If you cover vessels of any considerable size, in this state, with a bell glass full of water, at the end of fifteen days you may collect about 200 cms. of oxygen, containing traces of pure hydrogen and of carburetted hydrogen, probably resulting from the decomposition of the organic matters of the maceration by means of bacterian ferments.

There are other precautions on which it is useless to insist; the evaporated water should be replaced every ten or twenty days by water sterilised at 70° C. If the growth is to be continued the liquid will be robbed of its mineral constituents, and some drops of the before-mentioned solutions must be added—in this way the multiplication of the diatoms may be continued for two, three, or even four months. I have to-day in my laboratory a growth that, having been recharged two or three times, has been going on since December 5th, 1891, and which has always been healthy.

You may carry on in the same manner growths under the microscope, the only difference being that they must be of less volume—those which I use contain about 2 cms. of liquid.

Such are the principal things necessary to be observed in order to obtain, without difficulty, a growth of diatoms in fresh water. Side by side with these normal growths, others may be produced

in which purposely you give predominance to the physical and chemical elements; then the diatoms which grow under these conditions take strange forms, and I have given to these growths the name of "teratological growths." I have followed to the third generation these strange variations of form; among some of the *Nitzchias* and *Cyclotellas*, nothing is more curious than to see the first of these diatoms puffed out—symmetrically narrowed—and become altogether unrecognisable; while as to the *Cyclotellas*, so regularly constructed in a box-like form, you notice the valvular face lose the circular forms, become oval, triangular, quadrangular, or take the appearance of a closed curve, not angular, but very irregular; at the same time the flat surface of the circle is warped, becomes hilly—the ridges of the upper and under surface of the cylinders wander about forming hills and valleys—the general design of the *Cyclotella* remains, but presents an appearance of great modification. These modifications of form are clearly appreciated when noticed in progress, and it is not uncommon to see the box-shaped diatoms appear like accordions, as if, being flexible, they had been submitted to a strong pressure. This strange shape does not prevent these monstrosities being lively and perfectly endochromed.

The results obtained by the teratological growth appear to me very remarkable; they explain in the first place why we meet in nature with abnormal forms. It seems that if you could fix the diatoms in the strange forms I have referred to, you might not only produce an infinite variety of hybrids of the same species, but perhaps also be able to follow a series of modifications that might lead slowly from one species to another. I am only sure, at present, as to the possibility of producing by culture important changes of form in the siliceous carapaces of diatoms. I say no more on this subject, which is far too important to be treated here in an incidental manner.

MR. CONWAY, who is exploring in the Himalayas, finds the peaks difficult in their lower parts; the region above seventeen thousand feet is easy, but in bad weather one is cut off from the upper region by the next seven thousand feet below. There are numerous and vast glaciers descending to between eight thousand and nine thousand feet above sea level.

A Midwinter Month by the Mediterranean. Third Week.

BY G. H. BRYAN, M.A. Cantab.

PART III.—MENTONE.

IN the town hall, at Mentone, is the museum which M. Bonfils has formed, of archæological and natural history objects from the neighbourhood, and in the museum, when open, is M. Bonfils himself—an enthusiast, whose whole heart and soul are absorbed in the study to which he has devoted many years of his lifetime. The room is small, and M. Bonfils' only regret is want of more space; as it is, every available corner is crowded, and the objects cannot be well arranged. The colours of many of the Medusæ, as well as of the specimens illustrating the Flora and Algæ of the neighbourhood, have been most beautifully preserved, and among the archæological curiosities, an old plan of Mentone in the 13th century, and several other drawings, together with a collection of coins, are among the most interesting. But *the* prize of the museum is the human skull which was excavated in 1884 in the course of an exploration of the "Bone Caves" of the Red Rocks, conducted by M. Louis Julien, of Marseille, aided by M. Bonfils. The body to which it belongs was found at a depth of about 20 feet below the floor of the cave, and is considered to be undoubtedly palæolithic, while its position indicated the probability of its having been interred there. The whole of the *débris* forming the floor was filled with signs of human occupation, such as burnt charcoal and ashes, broken bones of animals, flint implements, etc.

This treasure was the second human skeleton discovered in these caves. The previous one, M. Rivière's "l'homme de Menton," was unearthed in 1872, at a much smaller depth,* and is now safely lodged in the Natural History Museum at Paris. The "new cave man" was more unfortunate in its fate, for, as M. Bonfils narrates with tears in his eyes, "it was stolen from under his very eyes!" The workmen engaged on the excavations seem

* *Science Gossip*, 1873, p. 178.

to have picked a quarrel over its discovery, which ended in a scramble and general plunder, every man laying hands on what he could of the remains; thus the whole of this valuable treasure has been lost except the skull. Even this would not have fallen to the share of M. Bonfils had it not been by a fortunate accident shattered into fragments by a pick-axe before it was actually unearthed. The fragments have now however been put together again, and all that now remains of the "new man" is in the museum of Mentone.*

On the top of the ridge separating the Carei and Cabrolles valleys, is the monastery of the Anunziata, to which a good path rises rapidly from the town below. Close to the beginning of the path I found Jersey ferns (*Grammitis leptophylla*) growing in a bank, and near here several trap-door spiders had built their nests. On the sandstone slopes grow rosemary, *Cistus salviæfolius*, *Dianthus saxifragus*, and such plants. Just above the monastery the path winds through a plantation of pines and small shrubs, an excellent hunting-ground for insects at the right season. A little heather (*Calluna vulgaris*) was still in flower, but the white "Mediterranean" heath (*Erica arborea*) bore no blossom as yet. The roots of the latter shrub are much used for making the tobacco pipes known as "briar root," "briar" being a corruption of the French *bruyère*, or heath. About here I recognised plants of *Coris monspeliensis*, and *Lavandula stoechas*, not in flower, however; and a neighbouring olive plantation was carpeted with leaves of *Anemone coronaria*. The ground was wet and slippery with the recent rains. Instead of turning down into the Turin, or Carei Valley by the paths, I determined to strike for the Cabrolles Valley, and soon got to a steep slope of soft grey gault, of which there is much about Mentone. I made one or two attempts to clamber down the slippery hillside to the torrent bed, and was rewarded by finding plants of *Globularia vulgaris* (one of which I brought home, and it is still flourishing), and the leaves of

* For a fuller account see the Report of the Aberdeen Meeting of the British Association in 1885. Since the above was written, three more skeletons have been unearthed in the caves, together with a lot of ornaments, flint implements, etc. These are described in the *Mediterranean Naturalist* for July and in *Natural Science* for June, 1892.

Aphyllanthes monspeliensis (not in flower), also by the fine view of the rocks beyond St. Agnese. But on nearly reaching the bottom I found that I had to retrace my steps, for the heavy rains had so swollen the torrent that it was impassable, and progress on my side of the stream was stopped by the slippery gault, which was weathered away into a precipitous slope, offering no foothold, and extending right up to where I had started down. So there was nothing for it but to turn back and clamber all the way up again, after which I turned down into the Carei valley, and back by the Turin road, finding on the way another plantation carpeted with *Anemone coronaria*, and one of the small "bloody-nosed" beetles (*Timarchia sempolita*).

My next walk was to Ciotti, one of the finest walks in the East Bay, the village being high up in the rocky gorge which runs down to the Pont St. Louis. After crossing this bridge, and gathering some flowers of the *Lavatera maritima* near the roadside, I turned out of the Ventimiglia road, just opposite the Italian custom-house. The path skirts the side of Dr. Bennett's garden, and in a sheltered corner by its side was a fine bush of the large *Euphorbia dendroides* in flower, of which I collected specimens; under the adjoining lemon trees were the leaves of *Gladiolus segetum*. Soon the village of Grimaldi was reached, from which the princes of Monaco derive their name, and then a steep climb through the olive groves, up a rough and stony path, brought me out on the open hillside, where grew several bushes of *Cneorum tricocon*, with small yellow flowers, and leathery oblong leaves; also *Dianthus saxifragus*, *Helianthemum roseum*, and near by, *Polygala nicæensis* (a near relative of our common *P. vulgaris*). Turning to the left, the path leads round into the rocky gorge, whence there is a splendid view over Mentone—a favourite subject for photographs and sketches. The fine, bold rocks on either side form a V-shaped frame, with the point of Mentone in the centre. Looking inland, a wild mountain valley presents itself, with its lower reaches clad with olives, and its further end losing itself in jagged peaks far beyond the Berceau; a short distance up is the village of Ciotti, with its church perched on an adjoining eminence.

A little below the path is a watercourse, carried round the side of the gorge to irrigate the lemons and drive an oil-mill in Grim-

aldi, and this in one place skirts the brink of a precipice. Like other similar water-courses and aqueducts about Mentone it is probably of some antiquity. Turning down the rocks I found several fine shrubs of *Coronilla emerus* in full flower, and one flower of *Campanula macrorhiza*. In the stream grew a kind of *Nitella*, while just below were thick festoons of maiden-hair fern (*Adiantum capillus veneris*), their large pinnæ in many cases more or less deeply cleft. A path, about a foot wide, goes along outside the little stream, but it was too muddy and slippery to allow of venturing far round the cliff.

Among the olives higher up I found dry seed vessels of *Nigella damascæna*, and some unusually fine plants of the "rusty-back" (*Ceterach officinarum*) in the walls, having larger fronds than some of the finest Somersetshire specimens. Above the village of Ciotti, and near the church, the nummulitic limestone crops up. The paths are in some places thickly strewn with loose nummulites of all sizes, ranging from about one-eighth of an inch in diameter to the size of a penny. It is also possible to find specimens split across, and showing the internal structure of the cells very well, though these are rare. Most of the nummulites are too brittle to allow of good sections being made for the microscope, and an examination of the broken fragments resulting from an attempt to grind down a thin section, suggests that they have undergone considerable changes in the course of fossilisation. Traces of coral structure may also be found.

The view from the village church extends over towards Monaco, to the Esterels beyond Cannes, and in the opposite direction down a wild valley, with La Mortola far below. Here for a short time I noticed a very curious effect. The sky was grey and cloudy, but an opening in the clouds allowed a beam of sunlight to fall on the sea behind Cap Martin, forming a bright round patch, which had a very weird appearance. From this point I returned down the hillside, and found the path in places entirely paved with nummulites, of sizes varying from that of a shilling to a penny.

My next walk was to Ste. Agnese, for which I took the whole day. The usual way ascends the ridge on the left side of the Cabrolles or Boirie valley, by the right of a small branch valley,

called the Val Solitaire. A steep climb brought me on to a ridge covered with such brushwood as small pines, cistus, rosemary, myrtle, *Spartium junceum*, and *Genista horrida*. At the right season this is an excellent entomological hunting-ground, but now there were only a few belated Red Admiral butterflies (*V. atalanta*) flying about, besides one large migratory grasshopper (*Pachytylus migratorius*), and a *Xylocopa violacea*, both of which I missed. The path winds in and out, affording alternate views of the Val des Chateigners and of the Gorbio valley, with the village of Gorbio at its head, and at last it rises in steep zigzags up the rocky mountain side. Up to this point the village of Ste. Agnese is quite concealed by the mass of rock in front, which effectually screens it off from view from the surrounding neighbourhood. At last the village is seen, picturesquely perched on the back of the crag, while down below the Val des Chateigners lies spread out. Another steep climb beside a little stream leads to the quaint old village, many of whose inhabitants live and die there, spending all their days within sight of the sea, but never leaving the village even to go down to the shore. Another climb leads to the top of the rock, on which is perched the old ruined castle of Ste. Agnese, at an altitude of 2,300 feet, 300 feet above the village. It is supposed to have been built by the Saracens. The walls are very high and massive, and the large windows, with the blue sky showing through them, are distinctly seen from Mentone, $5\frac{1}{2}$ miles away. Through the same windows there is a view over the wide expanse of valleys running seawards at our feet, bounded by the Berceau and other high mountains, while to the north stretches a bare and rocky valley—a continuation of the Cabrolles valley, between the sharp peaks of the Aiguille, still sprinkled with snow, and another mountain, while a snow-clad peak is seen further back. In the valley, some hundreds of feet below, a small waterfall is seen. On the rocks about the castle grow shrubs of *Juniperus phœnicea*, *Alyssum halmifolium*, *Asplenium trichomanes* and *ruta-muraria*, and *Ceterach officinarum*; I also found one small violet in flower. This was the only place near Mentone where I found the wall rue fern.

From the village I returned to Mentone by a wide path zig-zagging down the front of the rock. The descent is easy, but

stony, the path carefully avoiding all precipices. From lower down a projecting pinnacle is seen on the left of the main rock, very much resembling the Pillar Rock in our English lakes, but tinged with the characteristic red colour of the Jurassic limestone about Mentone. On the slopes near here grew shrubs of *Cneorum tricoccon*, also plants of *Helianthemum vulgare*. My way was next down a steep slope of loose grey gault, and was anything but good. In one place there was an abundance of long conical fossils, which looked like the shells of a kind of *Nerinea*. Soon I reached a tiny little wayside chapel, called Santa Lucia, whence the path followed a pretty ridge separating the Boirie valley from the Vallée des Chateigners. This is covered with the usual growth of scrub, and on the Junipers jelly-like masses of the fungus, *Podisoma fuscum*, occur. Just before emerging at the pottery I found some fine acute forms of *Asplenium adiantum nigrum*.

This was the first sunny day after the spell of rain, and in the evening the full moon was very bright. So still and warm was the air, that, although it was only the 5th of January, one could sit out in the hotel garden, in the moonlight, and enjoy the scent of the heliotrope, roses, and other flowers now in full bloom.

The following morning I hired a bicycle to ride to Monaco ; but I soon regretted I had not trusted exclusively to "Shanks' pony." The road was much more hilly than I had imagined. I nearly managed to ride to the top of the hill behind Cap Martin, but the next hill was considerably steeper, and with a hot sun shining down overhead, and the heat reflected by the rocks behind, riding was too much of a good thing. The last straw but one was when I came to a hill too steep to ride down. The last straw was when I got near Monte Carlo. Here there are any amount of dogs about the streets, and these seem to delight in worrying the cyclist as they come barking round, and are a perfect nuisance.

Both Monte Carlo and Monaco are getting very much overbuilt ; almost every square inch is covered with houses and hotels, many of them decidedly second rate, and the few available spaces a little further on bear the notice of *Terrain a vendre*. Along the roads were the triumphal arches and other decorations put up to welcome the Prince of Monaco on his return to the principality

the following day. Arrived at the station at Monaco I had had quite enough cycling experiences, so I put my machine on the next train, and rode back on a corridor carriage, whence I saw the scenery far better than on the bicycle, and the red-tinged rocks and deep blue sea, reflecting the sparkling sunshine, appeared in their full beauty as the train leisurely steamed along to Mentone. I saw one Bath white butterfly (*Pieris daphidice*) near Monaco.

In the afternoon I took the omnibus to the Quartier Garavan (a corruption of "Gare à vent," on account of its sheltered situation), and walked round the foot of the Red Rocks. At the base of these rocks are the Bone Caves, altogether six in number, where the human skeletons, already mentioned, were discovered, together with numberless flint instruments and bones of large quadrupeds; but the quarrying operations seem to have entirely destroyed the entrance of one of the caves. After passing the rocks, the path goes round a tiny little bay, and on to a small plateau, where grow fine shrubs of *Euphorbia dendroides*, and *Lavatera maritima*. *Cneorum tricocon* also occurs in one or two places. Among the loose gravel grew a few of the pretty pink *Convolvulus Cantabrica*, and some plants of *Fumana spachii*; both were in flower. A number of clouded yellow butterflies (*Colias edusa*) were flying about, but were difficult to catch. A curious kind of natural fountain is seen here when the sea is rough. The rocks form a kind of hollow cavern, with several openings in its roof, through which the waves project jets of water to a great height. The rock pools contain an abundance of the Peacock's Tail Sea-weed (*Padina pavonia*), and other algæ, besides innumerable small crustaceans. The scrapings from the sides of these pools are rich in diatoms. In a single "boiling" of the material I found specimens of *Biddulphia pulchella* (abundant), *Amphitetras antediluvianus*, var. *excavata*, *Rhabdonema Adriaticum*, *Synedra superba*, *Cocconeis punctatissima*, *Grammatophora marina* and *serpentina*, besides representatives of the genera *Triceratium*, *Asteromphalus*, *Eupodiscus*, *Campylodiscus*, *Navicula*, *Pleurosigma*, *Surirella*, *Actinosphenia*, etc. Returning by the Red Rocks I noticed several plants of *Matthiola incana*, all out of reach.

Before sunrise the following morning Corsica was distinctly visible, the long line of mountains, distant about 80 miles, stand-

ing out very sharp and clear against the horizon, until they appeared to be melted away by the rays of the rising sun.

A few hours later I took the train to Nice. In passing I noticed considerable changes had taken place at Beaulieu. It was such a pretty place twelve years ago, and now it is much built over! From Nice I started back to Mentone by the Route de la Corniche. The road, immediately after crossing the nearly dried up bed of the Paillon, begins to ascend the side of Mont-Gros, and soon gets clear of the town. Here I saw a plant of *Campanula macrorhiza* in flower high up out of reach, and several of the pretty pink *Anemone stellata*, just coming out. Where the road makes a wide bend I took a short cut over the hill behind the observatory, and here a child from a cottage brought out flowers of the purple *Anemone coronaria* and *A. stellata*, for which I gave her a sou. Regaining the road after a stiff climb, there was a fine view over the long line of snow-clad Maritime Alps, up the valley of the Paillon. A turn of the road brought us to a view over Beaulieu and the long promontory of St. Jean, stretching out into the sea many hundreds of feet below. To the far west the point of Antibes, the Lérins islands off Cannes, and the Esterel mountains were lit up by a blazing sun. A little further on a wall of jagged rocks, far below, forms the back of a red cliff facing the sea, and called the Petite Afrique. The road winds in and out of the mountains at a height of about 1,700 feet, but the telegraph wires take short cuts, and fall in a catenary across one of the depressions.

Several fortresses have been built on the adjoining eminences. A little further on the quaint village of Eza is seen, perched on a rock crowned by the remains of a Saracenic stronghold, at a great height above the sea, but far below the road. Near this point I found one piece of the yellow broom (*Spartium junceum*) in flower, although its proper time of flowering is not till about May. In a wet place by the roadside grew the pretty bog pimpernel (*Anagallis tenella*), and on the Jurassic rocks further on grew *Lavatera maritima*, *Cneorum tricocon*, and fine bushes of *Euphorbia characias*. From this point La Turbia is seen, with the remains of its fine Roman tower, built by Augustus Cæsar, but which was partially blown up by Napoleon. After passing the

little village of Turbia, the road gradually descends along the face of the cliffs, affording glimpses of Monaco, far below. Next it passes below Roquebrune, a village remarkable for the way in which the houses have been built into and among several gigantic masses of conglomerate, which must have rolled down the hill from above. From near here I saw Corsica again shortly after sunset, this time fainter than in the morning. Soon the base of Cap Martin was reached, and then Mentone, at a total distance from Nice of about 18 miles.

The following morning Corsica was again visible at about 7 a.m. In the afternoon I walked up the Cabrolles valley, or Val de Boirie. The carriage road then stopped at an oil mill, and the path followed the right bank of the stream till it crossed over to the village of Cabrolles by a rustic stone bridge. On wandering about the lemon and olive plantations near here, I found some fine trap-door spiders' nests, constructed by *Nemesia manderstjernæ*, a spider which not only excavates a tube, lines it with silk, and furnishes it with a flap door, but also adds an upward branch some way down, separating it from the rest with an inner door, so constructed that it can close off the whole lower portion of the nest against the intrusion of an enemy. Of plants I found a few capuchins (*Arisarum vulgaris*) still out, some fine fronds of *Asplenium adiantum nigrum*, var. *acutum*, one or two Jersey ferns (*Grammitis leptophylla*), and a quantity of plants of *Anemone coronaria* growing thickly over the terraces, but with no flowers.

The ridge flanking the valley on its left side near Mentone is called the Arbutus ridge, from the number of arbutus trees growing on it, and some enterprising genius has constructed a road from the valley below to the top of the ridge, ascending by a series of zigzags—a kind of Jacob's ladder arrangement, remarkable as a piece of engineering, but not as an object of beauty. It joins a footpath along the ridge, close by a hedge of beautiful roses, and on descending the ridge lovely views are obtained of Mentone, through the foreground of pines and arbutus trees. Here grew very luxuriant plants of *Dianthus saxifragus*, *Alyssum saxatile*, *Fumana spachii*, etc., all in full flower, and I also saw a Clouded Yellow Butterfly flying about among the pines.

My last walk before leaving Mentone was to Gorbio. The road passes in front of the modern Alexandra Hotel, and while the views up and down the Gorbio valley are not wanting in beauty, the first part of the walk loses much of its interest from being along a wide and fairly level driving road, constructed before 1877. A lemon plantation some way up is well known to visitors for its scarlet anemones, both single and double (*Anemone hortensis* and var. *pavonina*), which are much sought after by tourists when they are in flower. A little further on, after crossing the stream, the real climb begins. The carriage road executes a sweep of two or three miles round the slopes of the valley, but the old Gorbio road, a broad mule path, climbs straight up to the primitive village of Gorbio. The view higher up the valley displays a fine rocky chasm, the sides of which are tinged with the characteristic red hue before mentioned. I had no special "find" on this walk beyond a few daisy roots (*Bellis sylvestris*) and some trap-door spiders' nests; still, it was a most enjoyable ending to my fortnight's stay at Mentone.

BAROMETRIC PLANTS.—The *Petit Traité de Meteorologie Agricole*, by M. Cana, contains a list of prognostics *apropos* of the aspect which certain plants present according to the state of the atmosphere. The following are a few examples :—If the head of the gith (*Nitella sativa*) droops, it will be warm; if the head of the same plant stands upright, it will be cool; if the stalks of clover and other leguminous plants stand upright, there will be rain; if the leaf of the sorrel turns up, it is a sign of a storm; if the leaf of the willow grass slowly bends up, there will be a storm; if the flower of the convolvulus closes, it will rain; if the flower of the pimpernel closes, it will rain; if the flower of the hibiscus closes, it will rain; if the flower of the sorrel opens, it will be fine weather; if the flower of the same plant closes, it will rain; if the flowers of the carline thistle close, there will be a storm; if the flower of the lettuce expands, it will rain; if the flower of the small bindweed closes, look out for rain; if the flower of the pitcher plant turns upside down, it will rain, but if it stands erect, it will be fine weather; if the flower of the Cinque-foil expands, there will be rain, but if it closes the weather will be fair; if the flowers of the African marigold close, it will rain; if the scales of the teasel become close pressed against each other, it will rain.

The Microscope and its Accessories.

PART I.

TO the student of Nature who is not content to take the observations of others on trust, but would see for himself and subject such observations to a close scrutiny, the possession of a good microscope is essential ; but how shall the would-be observer determine, among the multitude of instruments, each of which is set forth by its constructor as the most effective—which is the one that most nearly meets the requirements—which of them is best adapted for the class of work he is contemplating—and which of them, in the great matter of cost, comes within his means ? This latter point is one of the greatest importance, for if cost is no object, there is little need of selection. An unlimited order to any of our first-class opticians will provide an instrument, with appliances, that will do almost anything, and will take part of a lifetime fully to understand and effectively to work. We have, therefore, thought that we should be doing good service to the cause of original research by passing in review such instruments, of our principal makers, as are specially intended for students' work. We have no interest in recommending any special make, and shall endeavour to place all before our readers on their own merits, pointing out, where practicable, the peculiar advantages claimed for each form. In order to avoid appearing to give undue preference, we shall arrange our notices of the instruments of the various makers alphabetically, though it is very possible that we shall not be able to confine ourselves strictly to this plan, for some special instruments may be brought under our notice which it would be unjust and impolitic to decline, because, being the production of Messrs. D., it had not been received till after those of Messrs. K. had been noticed.

It may be well to state that in the production of this series of articles we are favoured with the assistance of a gentleman thoroughly conversant with all the details of microscopical technique. In the present paper we shall describe

SOME STUDENTS' MICROSCOPES.

C. BAKER.—Last year Messrs. Baker brought forward a very convenient form of student's microscope, illustrated by Fig. 6.

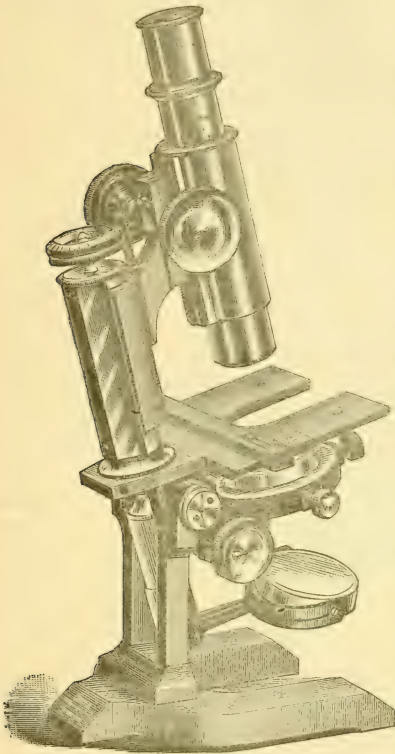


Fig. 6.

The instrument is supported on two columns, which arise from a horse-shoe foot. It has a rack and pinion coarse adjustment, a differential screw fine adjustment, and a draw-tube. The stage is of the horse-shoe shape, as advocated by Mr. E. M. Nelson, and it is provided with a Wright's finder; this will be found to be a most useful addition. The sub-stage carrying the condenser is worked by a rack and pinion adjustment. It is moreover provided with centring screws.

Instead of the horse-shoe base, this instrument can also be supplied with a tripod foot, which form of base is certainly to be preferred. Fig. 7 illustrates the same makers' stand of their "Nelson"

model series. As can be seen by the figure, the instrument is on a tripod foot, and has a rack and pinion coarse adjustment, differential screw fine adjustment, and a draw-tube. It has a horse-shoe stage with sliding-bar, but if preferred a circular rotating stage can be substituted. The sub-stage can be had either with or without centring movements.

R. AND J. BECK.—This firm supplies a great variety of expensive stands and is largely patronised by students. Fig. 8 illustrates their "Pathological" Microscope, as represented in the new edition of "Carpenter on the Microscope." It will be noticed that

the foot of the stand is a flat tripod, with a single pillar supporting the instrument arising from it. The peculiarity about this stand is the fine adjustment, which is placed below the stage, the actuating milled head being behind the column that supports the body-tube. It has a rack and pinion coarse adjustment and a draw-tube. There is a moveable glass stage, to which the object is affixed by spring clips. An achromatic condenser, diaphragm plates, and two eye-pieces are supplied with the instrument.

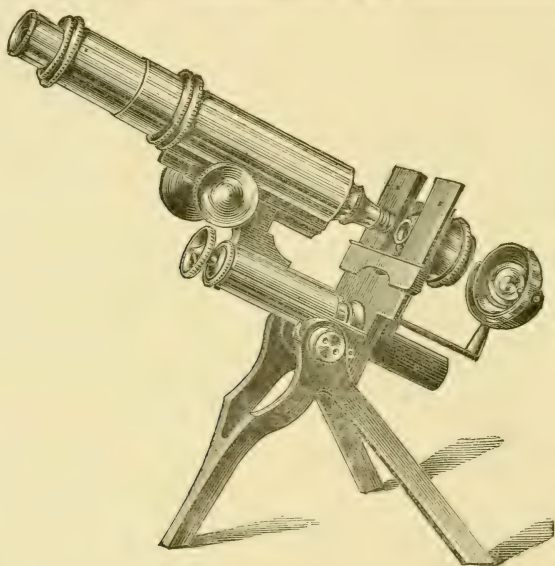


Fig. 7.

In the various forms of the "Star" Microscope, Messrs. Beck supply a useful class of stand at a ridiculously cheap rate. One of their latest additions to this series is the "Bacteriological Star," Fig. 9. This instrument is made in two forms: one with a sliding tube and the other with a rack and pinion coarse adjustment. It is almost needless to say that for all purposes a rack and pinion movement is by far the best. The fine adjustment is by a micrometer screw, the milled head of which is on the top of the pillar of the stand. The instrument has a draw-tube, spring clips to the stage, and a swinging double mirror. The special point in this stand is the sub-stage, which is on a screw

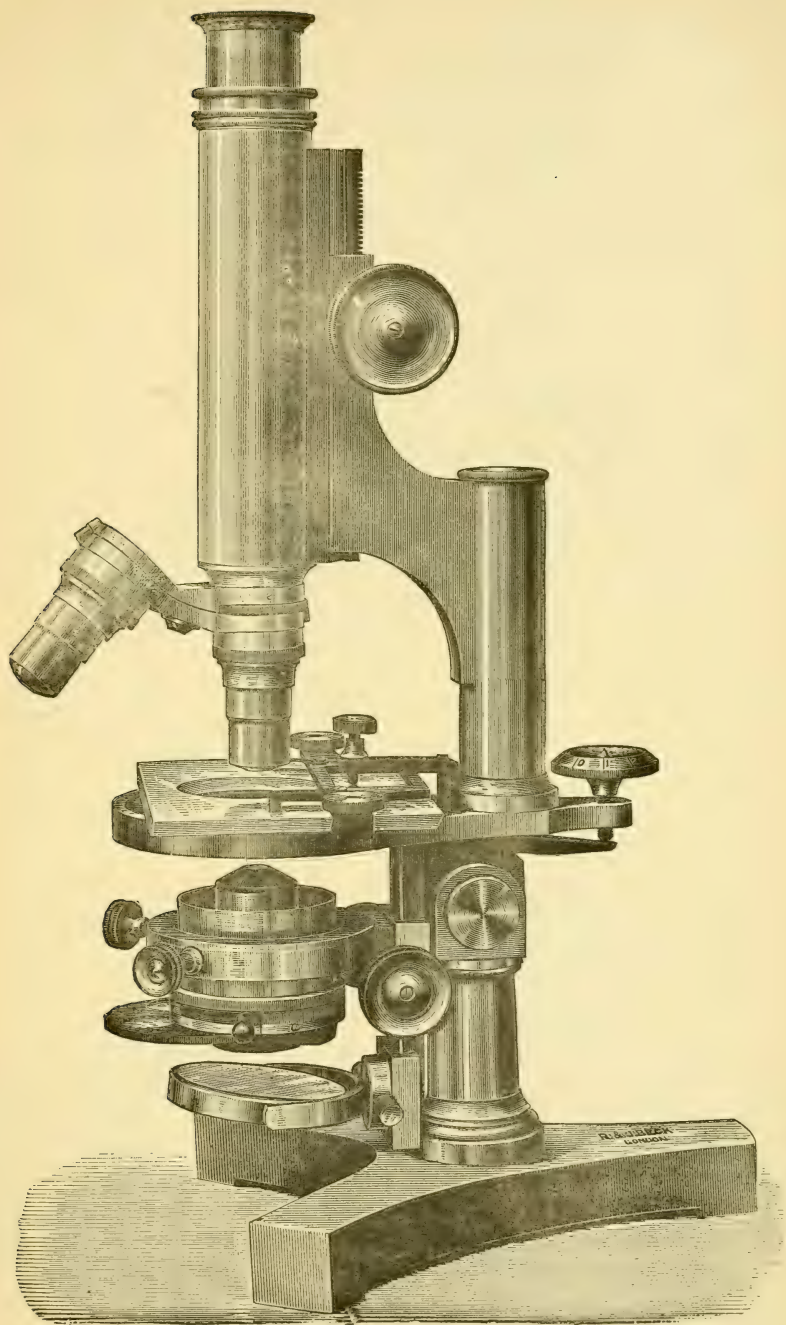


Fig. 8.

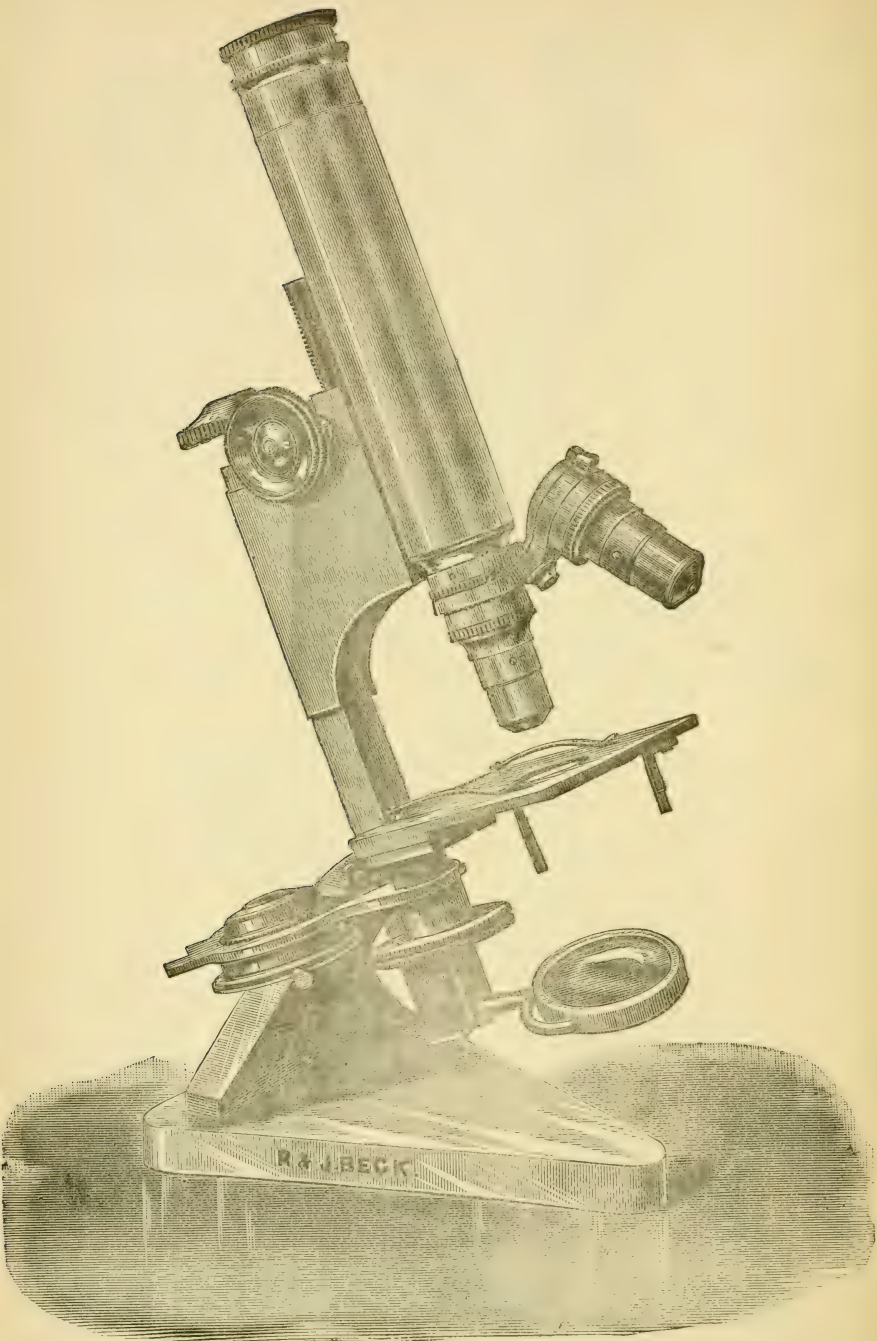


Fig. 9.

movement. It is raised or lowered by a milled head on the right-hand side of the stage, and when at its lowest position a further turn of the milled head throws the whole sub-stage out to the right-hand side, as illustrated, thus allowing the condenser to be easily removed or altered. The stand is sold with an Abbé condenser, iris diaphragm, and two eye-pieces.

W. JOHNSON AND SONS.—Fig. 10 illustrates Messrs. Johnson and Son's Advanced Students' Microscope. The instrument has a horse-shoe foot, the body being slung between two pillars. It has a rack and pinion coarse adjustment, differential screw fine adjustment, and a draw-tube, which is marked for the English and Continental objectives. The speciality in this instrument is the sub-stage adjustment, the milled head, A, of which is let into the pillar of the stand. This instrument was shown at the Royal Microscopical Society last year, when the sub-stage adjustment was highly commended by the late Mr. John Mayall, jun., who said that "it seemed to him that Messrs. Johnson had undoubtedly 'scored 1' by bringing out this screw-focussing adjustment for the sub-stage." A mournful point of interest is connected with this stand, it being the last instrument that Mr. Mayall criticised publicly. The sub-stage, which has screws for centring purposes, carries an Abbé condenser, with iris diaphragm. The sub-stage is fixed to the tail-piece by a bayonet fastening, and can be removed if occasion requires (see B).

POWELL AND LEALAND.—For the dearer class of stands a foremost place is occupied by Messrs. Powell and Lealand's No. 3 model, Fig. 11. This instrument is made on the same principle as their famous No. 1 model. It is supported by a tripod stand. The coarse adjustment is effected by a triangular bar, manipulated by two large milled heads. At the top of the bar is the arm which carries the body-tube. This arm also forms the case for their delicate fine adjustment screw, which acts directly upon the nose-piece; the actuating milled head of the fine adjustment is seen at the end of the arm. The stage has the ordinary rectangular and sliding movements. The sub-stage is fitted with mechanical centring movements, and has a rack and pinion coarse adjustment. It will be noticed that the plano-convex mirrors are fitted upon a double-jointed arm.

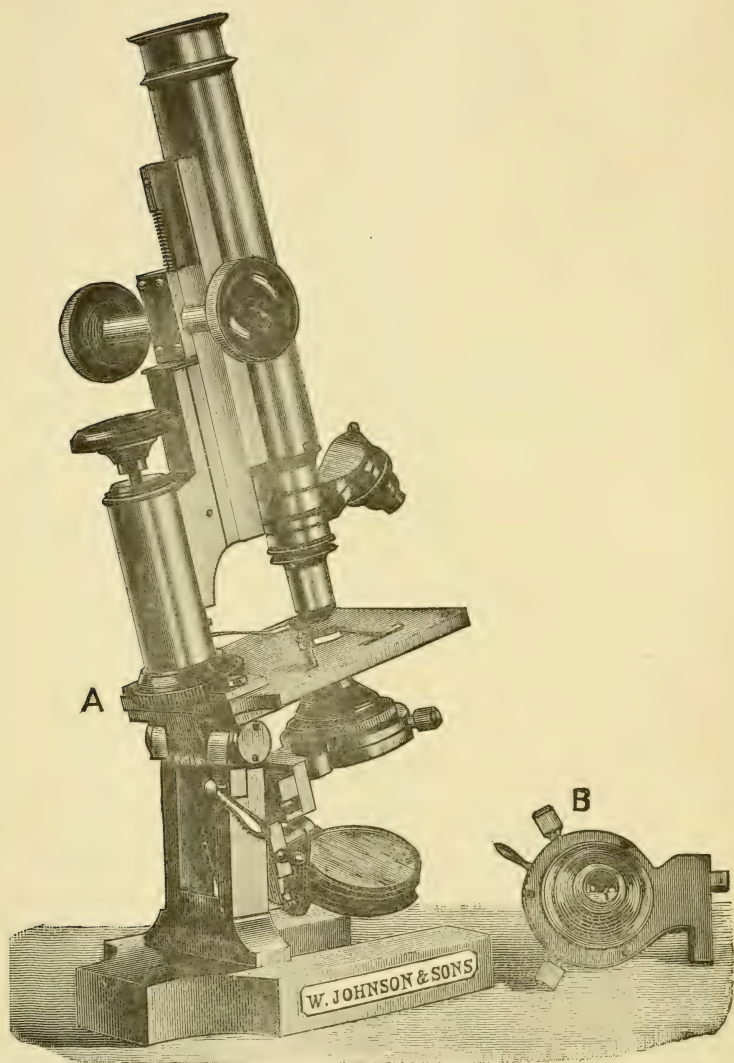


Fig. 10.

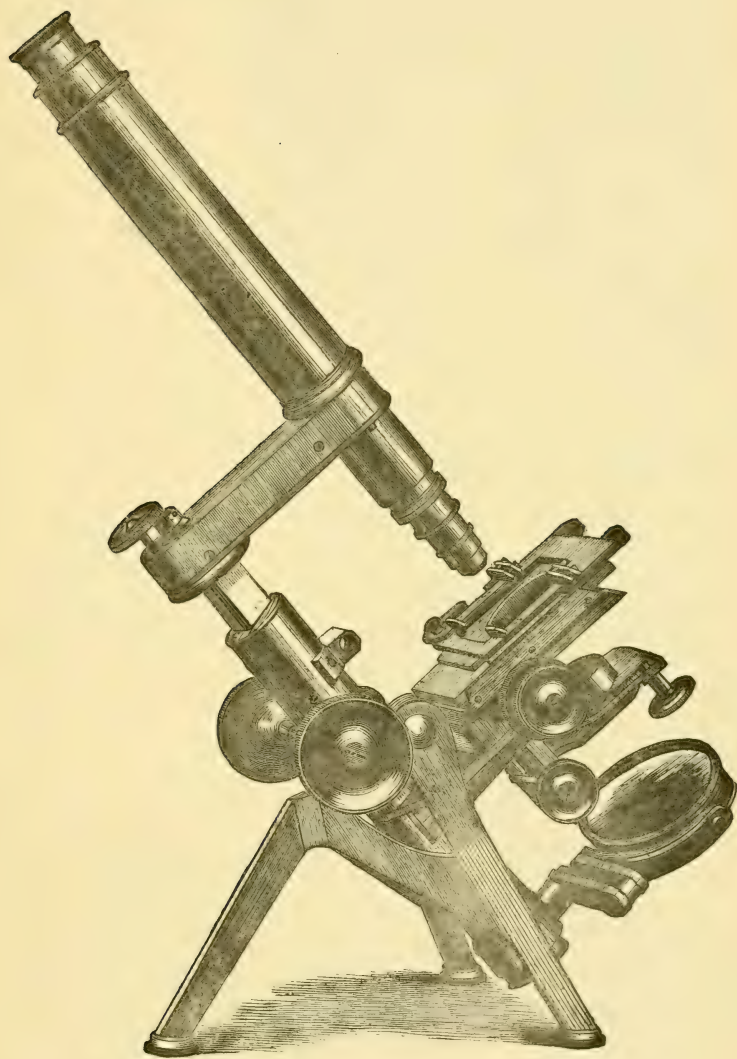


Fig. 11.

ROSS AND CO.—The excellence of Messrs. Ross's instruments, considering the reputation of the makers, need not be enlarged on.

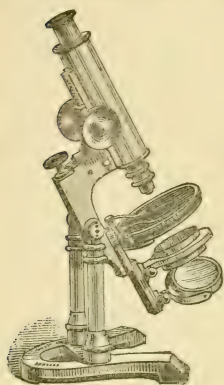


Fig. 12.

Fig. 12 illustrates their No. 4 stand. A general idea of the arrangement can be seen by glancing at the figure. This instrument is made on the lines of what is known as the Ross-Jackson model. The body of the stand is supported between two pillars arising from a "bent claw" foot. It has a rack and pinion coarse adjustment, a screw fine adjustment, a draw-tube, and a concentric revolving stage. The sub-stage apparatus is attached to a swinging tail-piece, thus being rapidly removable if direct illumination from the mirror is required.

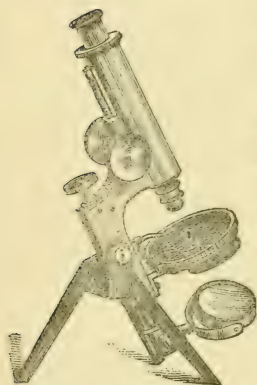


Fig. 13.

Fig. 13 is a somewhat similar stand, but supported on a tripod foot. This form of foot is greatly to be preferred, being far steadier than any other. The coarse and fine adjustments and the stage are the same as in Fig. 7; but there is no tail-piece for sub-stage apparatus. It has, instead, a brass collar affixed to the under-part of the stage, into which a revolving plate of diaphragms is inserted.

J. SWIFT AND SON.—Fig. 14 illustrates Messrs. Swift's Advanced Students' Microscope. The stand is supported between two pillars. The coarse adjustment is by rack and pinion, the fine adjustment being by differential screw. The body-tube is short enough to allow of Continental objectives being used, while the draw-tube will bring it up to the full English standard. It has a Nelson's horse-shoe stage, carrying a sliding-frame with spring clips. The tail-piece is grooved to receive a sliding bar, bearing a sub-stage fitted with centring screws (a rack and pinion adjustment can be had in place of the sliding movement). The sub-stage as

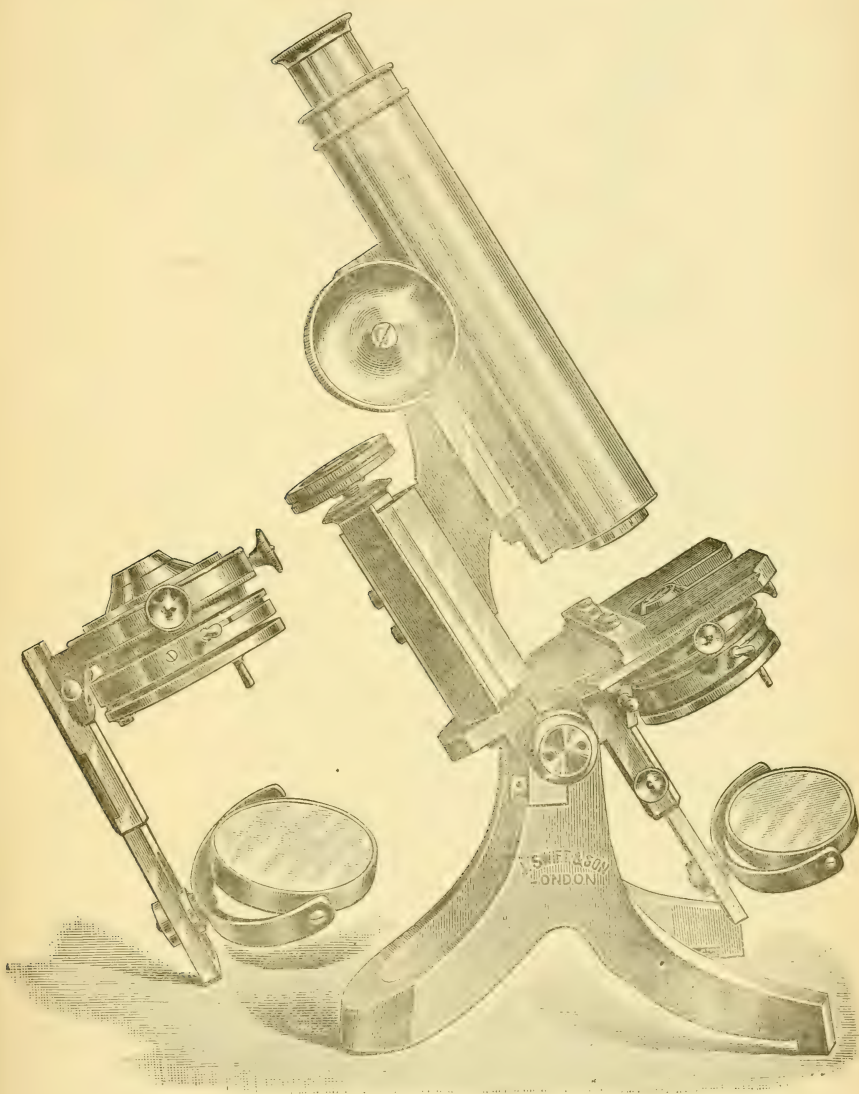


Fig. 14.

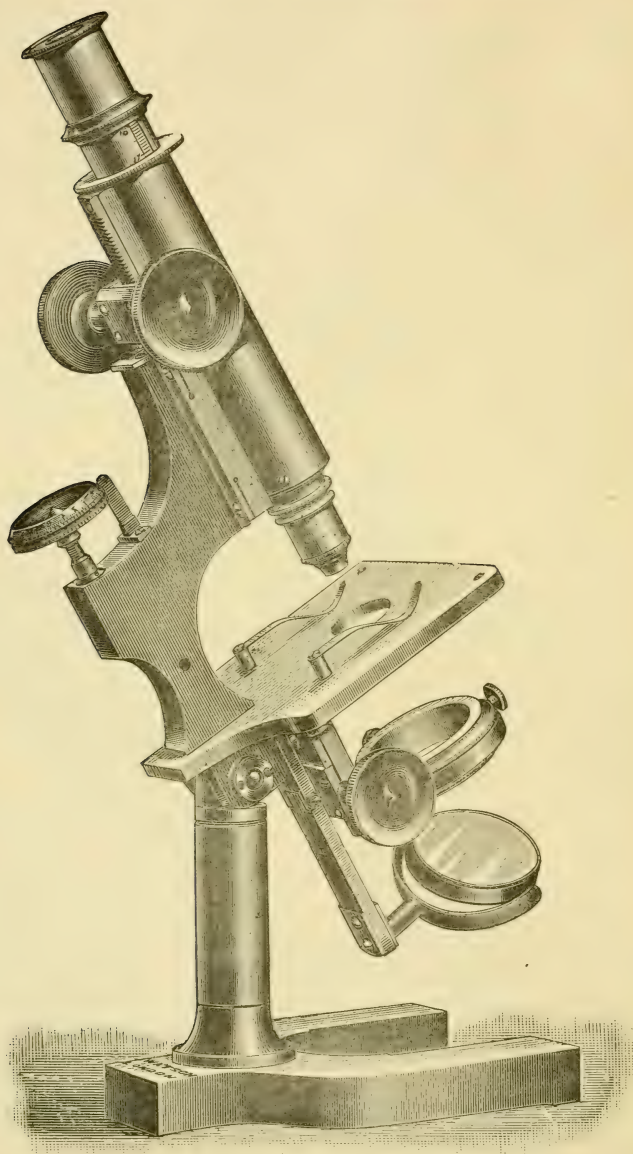


Fig. 15.

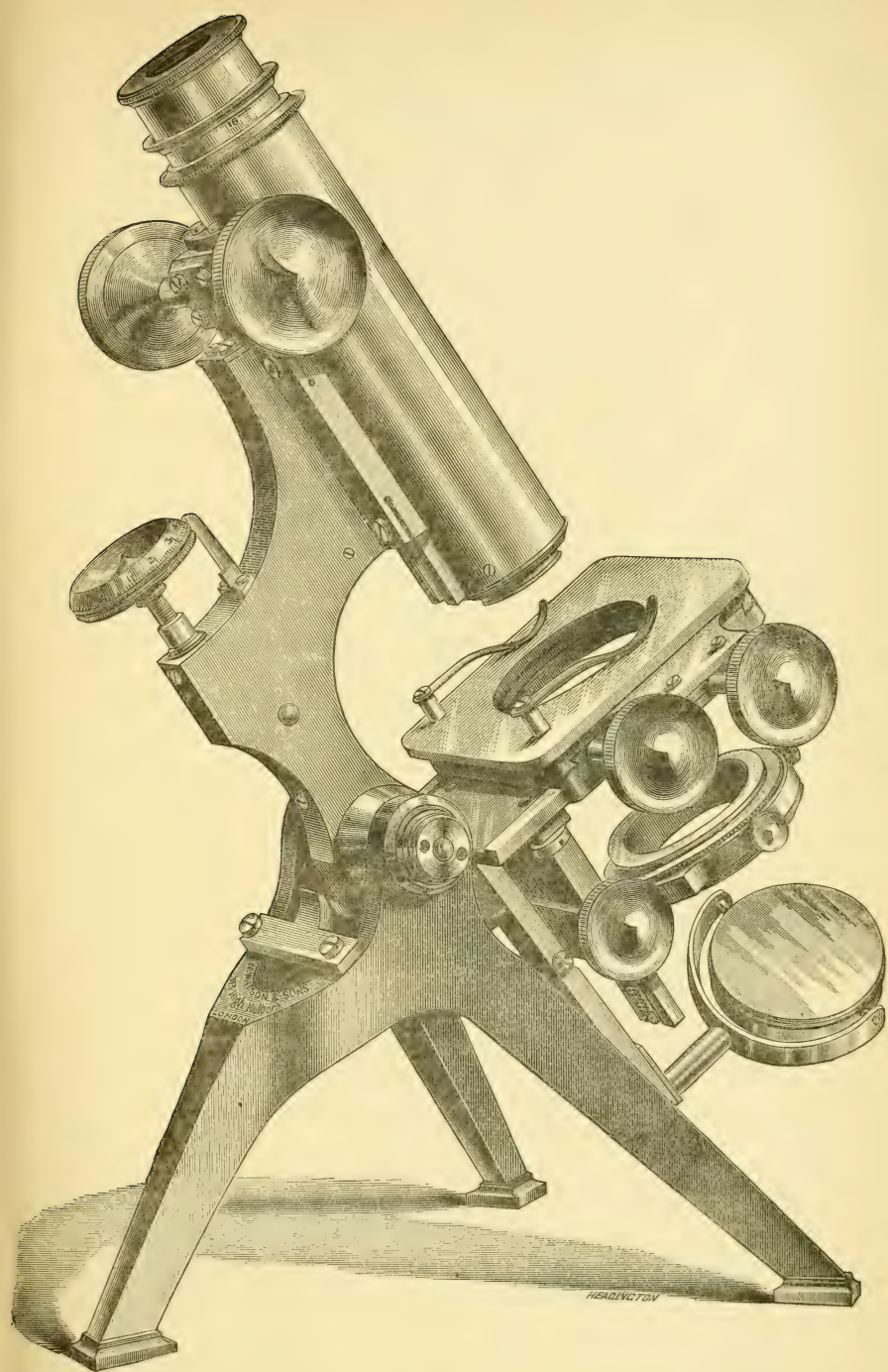


Fig. 16.

seen in the figure is fitted with an Abbé condenser. The swinging double mirror slides in a slot cut in the bar, and can easily be removed when required.

Messrs. Swift also supply a Petrological Microscope, built upon the same lines as their Advanced Students' Stand. It has a revolving glass stage with a divided edge. All the necessary polarising apparatus is attached.

W. WATSON AND SONS.—The "Edinburgh" Students' Microscopes of these makers represent a good class of working instruments. Fig. 15 illustrates their "Edinburgh" C model. The instrument is supported by a single pillar, arising from a horse-shoe foot. It has a rack and pinion coarse adjustment, a lever fine adjustment, and a draw-tube marked for the various objectives now in use. The sub-stage is worked by a rack and pinion movement. It is provided with screws for centring purposes and is mounted on a pivot, so that it may be turned on one side when direct light from the mirror is wanted.

The A and B stands of the Edinburgh series are much simpler than the C model, as, for instance, the A stand has a sliding-tube instead of rack and pinion coarse adjustment; it is also without the sub-stage, as represented in C. The B stand has a rack and pinion coarse adjustment, but is without the sub-stage. The D has, in addition to the movements of the C stand, a complete mechanical stage.

But as the Tripod form of foot gives a maximum of steadiness to the instrument, and Dr. Dallinger, in his new edition of "Carpenter on the Microscope," says:—"A broad base, resting on three points only, and these blocked with cork, is the ideal for a perfect instrument"; Messrs. Watson and Sons have constructed an instrument to meet these requirements. Fig. 16 shows Stand H of the Edinburgh Students' Microscope, made by Messrs. Watson and Sons. The original of this instrument was, as shown in Fig. 15, with the continental horseshoe base and pillar. The plainest of this series of instrument has only a sliding fitting for the coarse adjustment of the body, and an under-fitting to carry the Substage Condenser, so that a student buying only the plainest form of instrument can have the most perfect form of foot.

The microscope figured No. 16 has mechanical movements to the stage, with a very large rotating top plate, and a compound substage, with rectangular screws for centring and rackwork to focus. We consider this the most complete of their Edinburgh

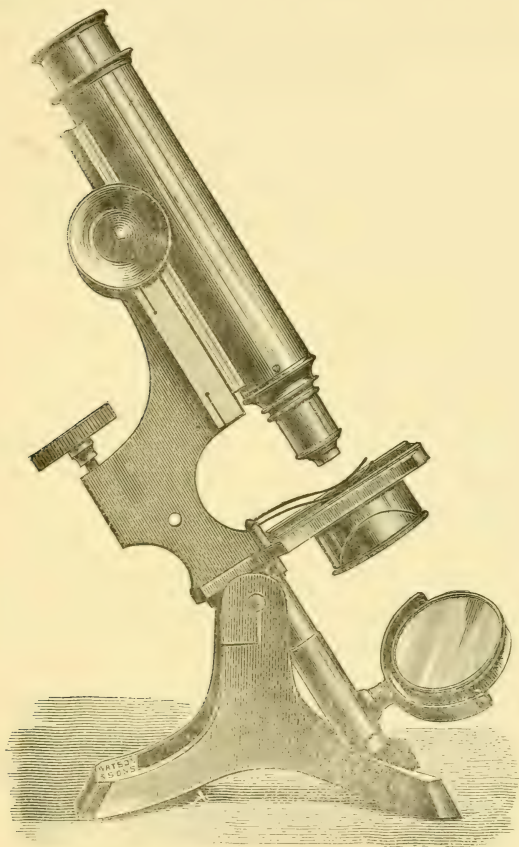


Fig. 17.

series. The makers also claim as a speciality their Fine Adjustment, which is of the lever form, it having compensating screws, by means of which the user can adjust the instrument for any slackness caused by wear and tear ; also the position of the milled head permits of manipulation with either hand, and as it does not

move with the body when attached to the connecting rod of a camera for photo-micrography, no alteration has to be made on the rod when using different powers.

Fig. 17 illustrates the Histological Microscope of this firm. This has a large stage, the continental length of body with draw-tube, and is made with a sliding body for focussing, or with rack-work as shown in the figure. It is provided with double mirror, and all fittings are of the universal size, so that any accessories may be used.

The Students' Petrological Stand of Messrs. Watson is built on a similar plan to their Students' Stand. No further comment need be made on them beyond stating that it has a revolving glass stage with divided edge and all necessary apparatus for petrological research.

It must not be considered that all the better class of students' stands have been here mentioned, nor that the instruments of other makers may not be quite as reliable. But these notes should only be taken as representing some of the more reliable instruments from a few of our London makers who *try* to keep pace with the demand for a working stand at a moderate price.

It will probably be noticed that the monocular form of instrument has been adhered to. This has been done for two reasons : one being that the monocular is cheaper than the binocular ; and the other, that with high powers better work and greater definition is got out of the single form than out of its double-barrelled and more costly relative.

Since writing the above, other forms of Microscopes have been brought to our notice, which we hope to describe on a future occasion.

IT IS SAID that a larger cave than the Mammoth Cave, situated on the Ozark Mountains, near Galena, Mo., has been explored for a distance of more than thirty miles. In it have been found bones of recent and prehistoric animals, including the hyena or cave-bear, and flint arrow-heads, but no bones of man. A few animals of the usual forms found in caves are still living there, including a white newt.

The Pollen of the Pine and Bean : A Comparative Study.

By H. G. WILLS, B.Sc., AND A. H. TROW, B.Sc.*

THE mature pollen-grains of both Pine and Bean consist of two nucleated cells—one known as the *generative*, the other as the *vegetative* cell. Both types of pollen-grain are microspores. Regarded morphologically, the vegetative cell is considered to be a reduced prothallium, and the generative cell a metamorphosised antheridium. The vegetative cell, from the physiological point of view, must be regarded as a polar body.

The two cells are enclosed in a common cell-wall, the outer layer of which is strongly cuticularised.

The chief differences between the two types may be exhibited thus :—

PINE.

- 1.—The pollen-grains possess lateral vesicles or wings.
- 2.—The vegetative cell is the smaller and the generative cell the larger.
- 3.—There is a permanent wall between the vegetative and generative cells.

BEAN.

- 1.—The pollen-grains possess no lateral vesicles, but only small projections.
- 2.—The vegetative cell is the larger and the generative cell the smaller.
- 3.—The wall between the vegetative and generative cells soon disappears.

Pollination is effected in the case of the Pine by means of the wind ; in the case of the Bean by bees and other insects.

In the Pine, at the time of pollination, the ovuliferous scales separate. The pollen-grains, blown by the wind, fall on the upper surfaces of these, and are guided by the projecting ribs of the scales to the apices of the ovules. Hence, by a curving upwards of prolongations of the integuments of the ovules, they are carried to the micropyles.

In the Bean, the stamens and stigma are so arranged that a bee visiting the flowers to obtain food, carries away pollen-grains from some stigmas to deposit them upon those of others.

The wings or lateral vesicles of the pollen-grain of the Pine increase its buoyancy, and so facilitate its dissemination. The grains are dry, easily shaken out of the pollen-sacs, and produced in enormous quantities.

* From the "Intermediate Science Directory."

The projections of the pollen-grains of the Bean facilitate their removal by bees. The grains are not easily shaken out of their pollen-sacs, are somewhat sticky, and are produced in, comparatively speaking, very small quantities.

The Influenza Bacillus.*

THE discovery of the germ of Influenza, first by Dr. R. Pfeiffer, and shortly afterwards by Dr. Canon, was announced on January 7th, at Koch's Institute, Berlin. Dr. Pfeiffer examined the sputum of Influenza patients, first sterilising and cleansing it by Koch's methods, and then treating it with Ziehl's solution or Loeffler's hot methylene blue. A large number of micro-organisms then became visible under the microscope, and it soon appeared that they were mostly of the same kind. This was always the case with the sputum of patients suffering from Influenza alone; when the disease was accompanied by other pulmonary disorders other bacteria were also present. It was intelligible that this bacillus had so long escaped detection, for it was far smaller than any micro-organism hitherto known, and the circumstance that its two extremities stained more intensely than the intervening parts gave them a striking resemblance to diplococci and streptococci. Pure cultures of the bacillus were cultivated. The colonies were so small, that at first they were only visible under a magnifying glass. Glycerine Agar proved the best nutritive medium. One of the characters of the bacillus was its immobility, the colonies not flowing together, but remaining separate. Monkeys, rabbits, guinea-pigs, rats, pigeons, and mice, were inoculated, but Influenza was only produced in the first two. Dr. Canon's method was to examine under the microscope blood from Influenza patients, taken during fever. Organisms hitherto unknown were found. Special attention was attracted to them, as they were found only in the case of feverish influenza patients and disappeared from the blood as soon as recovery took place. The examination of his discovery by Drs. Koch and Pfeiffer showed the bacillus to be identical with Pfeiffer's.

* *The Lancet*, 1892, Vol. I., pp. 169—170.

The Recent Sun=Spots.

SIR Robert S. Ball, Lowndean, Professor of Astronomy and Geometry in the University of Cambridge, has written an article for the November number of the "King's Own," in which he deals with the recent outbreak of Sun Spots. He says :

"At the present time (1892) it happens that the spotted area of the sun must be nearly at its maximum, even if it has not already reached it. It will thus be seen that we have now an opportunity of investigating solar phenomena, which ought not to be neglected by those who desire to learn something of the wonders of our great luminary. Perhaps some may ask what, after all, is the interest which attaches to such matters as sun spots? How can they possibly teach us anything, or what connection can they have with terrestrial matters? Here we happen to touch on a scientific question of very great interest about which, unfortunately, very little is at present known. It is quite certain that the presence of abundant sun spots does correspond in some remarkable manner with certain terrestrial phenomena.

Suppose that we take a mariner's compass of an especially delicate construction. Suppose that we hang the magnetic needle with such careful precautions that its slightest movement shall be perceptible. Suppose we carefully screen it from all external interference. Suppose we put it, not, indeed, in the cabin of a ship, which rolls about at the mercy of the winds and waves, but in the basement of a specially-constructed building, from which all iron is absent, because that metal interferes with the action of the earth on the magnet. Suppose that we further provide microscopes by which we are enabled to study with minute attention the slightest movement of the needle. Or, suppose that, with still greater refinement, we arrange a photographic apparatus by which the needle shall be made to record, with faultless accuracy, its exact position at each moment of time, then we shall be able to learn something of the connection between sun spots and terrestrial affairs.

We are accustomed to speak of the compass as pointing to the north, but it is not to be understood from this that the

direction indicated by the magnetic needle undergoes no changes. The fact is that it is in incessant movement. It is true that these movements are generally so small that they do not in the least interfere with the practical utility of the compass. In fact, such changes as those to which I am now referring would not be at all perceptible on an ordinary ship's compass; they would require the refinement of apparatus and observation which I have already indicated. But there is no doubt that incessant fluctuations of the needle are in progress by day and by night, and sometimes it will happen that what is known as a magnetic storm will take place. On such an occasion the needle is thrown into a state of oscillation, which may be described as violent in comparison with the movements which it has on more normal occasions. It has been shown by a careful study of upwards of a hundred magnetic storms, that there is an almost invariable connection between them and some disturbance of the sun's surface.

It is not at present easy to say what the precise character of that connection may be, but it is absolutely certain that whenever the sun is in a highly disturbed state, as shown by the sun spots and other solar features, that then there is a distinct disturbance in the magnetic state of the earth. Other similar phenomena can also be cited. Auroras are most usual when the magnetism of the earth is in unusual excitement, and auroras are seen in unusual splendour, and frequently at a time when the sun is in a state of agitation.

Special instances have also been noticed in which there is an absolute coincidence in time between the occurrence of some striking phenomena in the sun and a marked outbreak of magnetic phenomena on the earth. A very interesting instance of this is recorded by the distinguished American astronomer, Prof. Young, who, on the 3rd of August, 1872, perceived a violent disturbance of the sun's surface. He was told the same day by a gentleman who was engaged in magnetic observations, and who was quite in ignorance of what Prof. Young had seen, that he had been obliged to desist from his work in consequence of the violent fluctuations of the needle. On the same day a magnetic storm was indicated by the instruments in England, at a distance of many thousand miles from where Prof. Young was making his observation.

It seems that we have still a good deal to learn with respect to this matter. Perhaps we may liken the periodicity of the sun to the periodicity of the great geysers in Iceland. In the latter phenomenon there is a sudden outbreak of heated water, which occurs with some regularity in a certain number of hours after the last outbreak. There is a somewhat similar pulse in the sun, and, considering the fire of the body involved, it is not surprising that a period of eleven years should divide two successive outbreaks. But how it is that these solar phenomena should be able to set our magnetic needles into a tremble is a matter to which science has not as yet been able to give a quite satisfactory answer."

—*The Standard*, Oct. 20, 1892.

Microscopical Technique.

COMPILED BY W. H. B.

Glycerine-Mounting.*—Mr. C. E. McClung writes on the merits of glycerine as a mounting medium. He finds the advantages of glycerine are its being non-volatile, colourless, slightly affected by changes of temperature, has a high refractive index, and remains perfectly colourless for any length of time. "The glycerine should be pure and free from dust and *air-bubbles*. To keep it free from these contaminations, devices such as are recommended by Carpenter and Prof. James are excellent. These are bottles containing the glycerine and provided with glass tubes, whereby the glycerine is forced out by air-pressure. The cements may be of a balsamic nature, but preferably zinc oxide or asphalt. Any cement not affected by the medium may be employed, but experience has proven that the two above named are the best. The other essential parts of the completed mount are the slip and cover-glass. No special mention is made concerning these except that they should be *perfectly clean*. To ensure this, the practice of leaving them until ready for use in a bath of ordinary battery fluid is recommended. Upon the consistency of the cement depends, in a great measure, the formation of a good cell. It should not be thin enough

* *The Microscope*, XII. (1892), pp. 201—203.

to spread, yet should flow readily and smoothly from the brush. The depth of the cell should be such that a complete support shall be provided for the cover-glass without causing it to bear upon the object when cemented down, and yet should not be of such a depth as to interpose an unnecessary stratum of glycerine between the section and the cover-glass. Of more importance, perhaps, than any other point is the direction regarding the age of the cell. It is a common practice to ring a cell and use it while fresh, the manipulator arguing that a more perfect union of cell-wall and cover-glass is secured in this manner. *Perhaps* this is true ; but it is at the expense of the slide's usefulness. The author already quoted is authority for the statement that any ordinary balsam cell will, in drying, shrink 30 per cent. Under these conditions, and in view of the fact that glycerine is non-compressible, something must give way when the cell contracts, and this is either the cover-glass or the cell-wall. Whichever it is, the final result is the destruction of the mount and loss of all the work involved in its preparation. This leads us, then, to make the following statement :—*Never use a green cell.* The older the cell the better, and, at ordinary temperature, two weeks is the shortest space of time in which a cell of medium depth will become seasoned. . . . In placing the section care should be exercised to have it exactly in the centre of the cell. With the section thus situated, a drop of glycerine is allowed to fall upon it from the dropping-bottle. Take the clean cover-glass and place the left side in contact with the drop of glycerine ; draw it over until supported on the left edge of the cell-wall . . . and allow the cover-glass to fall gradually by supporting the right edge with a needle. Having thus placed the cover-glass and centered it, place a clip upon it. The superfluous glycerine thus forced out is washed away by means of a jet of water from the wash-bottle, so directed as not to strike the cover-glass. Some water *does* get under, but this does no harm, as it supplies moisture which the glycerine otherwise would have by 'creeping' from the cell. When *thoroughly* dried by means of strips of bibulous paper, the slide is ready for the last step—securing the union of cover-glass and cell-wall. This result is best obtained by ringing once around the cover-glass, and allowing this coat to dry before applying cement enough to

hide the junction of the cover-glass and cell-wall. When this latter step is accomplished, the mount is essentially complete, but no one who has a pride in his work will leave the slide unstripped. There is no more beautiful slide than one formed of white cement ringed with black. Properly labelled and cleaned, the slide is ready for the cabinet, and if the due amount of care has been exercised in its preparation, it will always be a source of pride and pleasure to its owner."

Notes on Celloidin Technique. —Mr. A. C. Eycleshymer gives the following as the result of his experience in working with celloidin. The prepared fragments of celloidin are placed in an air-tight chamber, a four-ounce wide-mouth bottle being very suitable for this purpose, and enough ether-alcohol (equal parts of acid-free sulphuric ether and absolute alcohol) poured in to cover the fragments. The ether-alcohol should be added until no celloidin remains undissolved. It should finally be of the consistency of very thick oil. This solution may be labelled No. 4. No. 3 consists of two parts of No. 4, diluted with one part of ether-alcohol; No. 2, by proceeding in a like manner with No. 3. No. 1 is a mixture of absolute alcohol and sulphuric ether in equal parts.

The object to be embedded is transferred from 95 per cent. alcohol to solutions 1, 2, 3, 4 successively, in each of which it remains from a few hours to days, depending upon the size and permeability.

In embedding, unless orientation is desired, the ordinary paper-box is best. A thin plate of lead is placed in the bottom and the embedding solution poured in. The object is taken from the same solution, and with needles wet in ether placed in the desired position. Fine needles may be passed through the box to support the object.

In hardening, Viallari's chloroform method is preferable. An air-chamber should be filled with chloroform. After the mass is thoroughly hardened—which requires about twenty-four hours—it is removed, the paper cut from the sides, and transferred to 70 per cent. alcohol for a few hours.

It is now ready for sectioning. Blocks are trimmed to fit the

* *American Naturalist*, XXVI. (1892), pp. 354—357.

clamp of the microtome. Solution No. 3 is poured over the block; into this the celloidin block is pressed, after dipping the under surface in solution No. 1. Place in chloroform until hardened.

Reconstruction points are often very desirable. For this purpose the ordinary metallic embedding box, made of two L-shaped pieces, held in place by overlapping strips, is used. The ends and sides are perforated in as many places as desired by a very small drill. The holes should be so drilled that the silk threads which are drawn through run parallel. After being drawn tightly, they are cemented to the sides of the box by a drop of celloidin. Five or six cm. of the thread should be left hanging. The bottom of the box is made by fitting in a piece of heavy blotting-paper. The object is placed upon the threads in the desired condition and the embedding mass poured in. As soon as hardened, the celloidin holding the threads is dissolved by a drop of ether. The loose ends are soaked in solution No. 2, which has been thickened by the addition of lampblack. The threads are then drawn through, leaving the lampblack adhering to the celloidin, thereby forming excellent reconstruction points.

For small objects, where reconstruction points are not needed, the following method may be advantageously employed:—The heads are clipped from fine insect-pins, which are then placed in handles in such a way that they may be easily removed. On these pins the objects are oriented in the desired condition. The pins are then removed from the handles and fixed in a cork, previously perforated by a somewhat larger pin. As fast as the pins carrying the objects are inserted the cork is replaced in the tube, which is filled with alcohol. A half-dozen fish or amphibian ova may be oriented on the same cork. If desirable to draw the objects *in situ*, a piece of lead may be pinned to the cork and the whole immersed in alcohol. The corks carrying the oriented objects are transferred successively to tubes containing the different solutions. When ready for final embedding, a piece of porous paper is wrapped about the tubes and cork and pinned. The cork is now removed, allowing the embedding solution to fill the paper tray thus formed. A lead is fastened to the cork and the whole placed in chloroform until hardened, after which the paper is cut from the

mass and the pins drawn through the cork, when it is ready for sectioning. This method offers many advantages in that several objects may be cut at the same time; drawings may be made after orientation; the objects are transferred from one solution to another more rapidly, etc.

In cutting, care should be taken that the knife is placed as obliquely as possible and kept constantly wet with 70 per cent. alcohol. For this purpose an ordinary pipette, provided with a large rubber bulb, is used. As fast as cut, the sections are drawn back on the blade of the knife by means of a needle, and arranged in a single row until the blade is filled. To remove these, a heavy paper spatula is placed directly upon the section, to which it adheres, and may be drawn off the edge of the knife and transferred to the slide. By slight pressure together with a rolling movement, the section is left in the desired position. Sufficient alcohol is kept on the slide to prevent drying, but not enough to allow the sections to float. When the requisite number have been arranged, they are covered with a strip of toilet-paper, which is held on the slide by winding it with fine thread. The sections being thus firmly held in position may be stained, etc. They should not be placed in absolute alcohol, but cleared in 95 per cent., in a mixture of equal parts of bergamot oil, cedar oil, and carbolic acid. When cleared, the excess of fluid is removed by a piece of blotting-paper. With gentle pressure, sections which are by chance loose are firmly fixed in position, the thread is now cut, the strip of paper rolled back, and balsam and cover applied.

If the object is stained *in toto*—which is often the case—much time may be saved by the following method:—The stained object is embedded in the usual manner, but after hardening in chloroform and removing the paper, the celloidin block is transferred to 95 per cent. alcohol for twenty-four hours, then to carbolic acid (Bumpus, *Amer. Nat.*, January, 1892, advises the use of thymol) or glycerine, in which it becomes as transparent as glass (Mr. Eycleshymer finds that the clearing mixture answers the same purpose as the carbolic acid, but requires a little longer time).

Orientation is now accomplished with the greatest ease. In cutting, the knife is wet with the clearing medium given above. The sections may be arranged in serial order on the knife-blade

until a slide-full is obtained, when they are transferred, and balsam and cover applied. By this method long series may be readily handled. Glycerine is used only when the mounting medium is glycerine ; in this case the knife is wet with glycerine.

Paraffin Infiltration by Exhaustion.*—Mr. A. Pringle finds that this system of embedding objects or tissues in paraffin is of value in ordinary work. The advantages claimed are :—Great celerity ; certain and complete infiltration ; certain removal of the solvent ; absence of distortion of the tissue elements ; obviation of necessity for prolonged heating of the objects ; possibility of using the same paraffin over and over again ; pecuniary economy.

The only apparatus required is a small, simple air-pump, with its usual glass chamber. The plate of the air-pump is smeared over with glycerine or lard ; over this is laid a sheet of india-rubber, which is also smeared with glycerine or lard. For greater convenience the air-pump should have near the plate, between the air-inlet and the plate, a tap, which is to be closed after the air is exhausted. If the paraffin-stove is large enough to admit the air-pump, with or without its barrel, so much the better. If no stove large enough for this is at hand, it may be well to remove the wooden stand on which the plate is usually mounted.

Any of the ordinary processes preparatory to the embedding are available, but preference is given to chloroform for reasons that will appear apparent as the process is described. The preparation is now put into the melted paraffin, the dish containing it is placed in the air-pump, and the air exhausted. So long as the bubbles rise the pumping may be continued, but it is well, after a little pumping, to let air into the receiver at least once. Of course, the paraffin is to be kept melted the whole time. If the stove will take the air-pump, the best way is to exhaust till the bubbles rise in great numbers to let in the air, to exhaust again, and, turning the tap suggested, to put the whole apparatus into the stove, where it may be left for a few minutes. The air is let in once more, the dish is removed from the pump, and put into the stove till the bubbles have disappeared from the surface, when the process is complete. After chloroform-preparation, the process takes fifteen

* *Journ. Pathol. and Bacteriol.*, I., 1892, pp. 117—119.

minutes. Benzole and cedar oil are not so easy to remove as chloroform, and it is necessary to remove all but a trace of the clearing substance, otherwise the paraffin will be soft and bad for cutting. The only modification entailed by the use of benzole or cedar oil is that it is well to give two baths of paraffin, exhausting during the first, but not necessarily during the second. This modification should not entail more than about five minutes' extra time, if the paraffin is melted ready for use. Mr. Pringle recommends an air-pump on the Tate principle, as it can be used for other important purposes. He does not find that exhaustion facilitates the hardening process, but thinks that further experiments are necessary to settle the point.

In the fixing, hardening, and embedding processes which he uses and now recommends, the tissue is fixed in saturated HgCl_2 (corrosive sublimate) for about twelve hours, washed in running water for a like time, and then put for twenty-four hours into 30, 50, and 70 per cent. alcohols consecutively, being kept in the latter until further steps are to be taken. Müller's fluid may be used in the same way, followed by alcohols as above. Then the tissue is placed in pure methylated spirit, absolute alcohol, and a second time in absolute alcohol, each for twenty-four hours. Then chloroform is placed with a pipette or syringe under the alcohol and left twenty-four hours. This mixture is then poured away and replaced by pure methylated chloroform, and the containing vessel is left, loosely stoppered, on the top of the paraffin stove or in any warm place for twenty-four hours, till any trace of alcohol has vaporised. The tissue is then placed in the melted paraffin, and the air-pump may come into use as soon as the tissue has warmed through.

Fixing Paraffin Sections to the Slide.*—Dr. G. L. Gulland uses a modification of Gaskell's method. The paraffin block containing the tissue must be trimmed very carefully, care being taken that the surface meeting the razor is exactly parallel to the opposite surface, and that the block is exactly rectangular. A thin layer of soft paraffin is then applied to the surface meeting the razor and to the opposite surface. This is best done by dipping these surfaces

* *Journ. Anat. and Physiology*, xxvi. (1891), pp. 56—59.

into the melted soft paraffin, and when this has become firm the surfaces are again trimmed square. The reason for this very special care is that any curve in the ribbon of sections produced by neglect of this precaution is accentuated by the flattening out of the sections. The ribbon is then divided, and one end is seized with forceps and the other end is gently lowered on to the surface of the warm water. When the flattening is complete, a slide is immersed in the water, and the ribbon is floated to its position with a stiff brush. As much of the water as possible is then drained off, and the rest evaporated by placing the slide on the top of an oven where the temperature is just below the melting point of the paraffin. When the water has evaporated completely, the opacity of the sections disappears, and they become much more transparent and look dry. When the fixation is quite complete, the paraffin is melted by putting the slide inside the oven for a little while, and is then washed off with turpentine or xylol. One of the great advantages of this method is the perfect ease and safety with which it allows sections on the slide to be manipulated, so that the most various stains and reagents can be applied successively to a slide. Of course, a single section can be mounted in the same way, and, when desirable to examine a few sections with as little delay as possible, warm methylated spirit, or even absolute alcohol, evaporate more rapidly than water, while the fixation is as perfect with them and the method of use exactly the same, as with the less volatile liquid.

Method for making Paraffin Sections from Preparations stained with Ehrlich's Methylen-Blue.*—In the course of his work Mr. G. H. Parker found it necessary to devise a method for making paraffin sections from preparations in which the nervous elements had been stained with Ehrlich's methylen-blue, of which he now gives the following account :—"In order to stain the elements in the nervous system of a crayfish, 1/10th to 1/20th ccm. of a .2 per cent. aqueous solution of methylen-blue was injected into the ventral blood sinus, the animal afterwards being kept alive in a glass aquarium.

"In about fifteen hours many of the ganglion cells and nerve-

* *Zool. Anzeiger*, xv. (1892), pp. 375—377.

fibres in the peripheral as well as the central nervous organs were stained intensely blue.

“Preparations made in this way, after being removed from the animal, retain their colour only about an hour, but, as is well known, they can be made more nearly permanent by treating them with reagents, which precipitate the methylen-blue, such as picric acid, ammonium picrate, potassic iodide, potassic ferro-cyanide, chromic acid, or corrosive sublimate. Of these reagents, the one last named, in addition to being an excellent fixing reagent, yielded the most satisfactory precipitate. In a well-stained ganglion or nerve, a cold, concentrated, aqueous solution of corrosive sublimate converts the methylen-blue into a finely-grained purplish precipitate.

“In order to bring such a preparation into paraffin, it must first be dehydrated. The dehydration cannot be accomplished by the use of alcohol, for this fluid dissolves the precipitated colour. As a substitute for alcohol, two fluids—aceton and methylal—were tried. In aceton the precipitate is as soluble as in alcohol, and in pure methylal it is also slightly soluble; but in methylal containing some corrosive sublimate it remains unaffected. The tissue was, therefore, dehydrated in a solution composed of 1 gramme of corrosive sublimate and 5 ccm. of methylal.

“The preparation, after being dehydrated, is, of course, permeated with a strong solution of corrosive sublimate in methylal. To free it from corrosive sublimate and replace its methylal gradually with xylol is the next step. This is in part accomplished by putting it next into a mixture composed of two parts xylol, one part pure methylal, and one part of the dehydrating mixture of methylal and corrosive sublimate. In this mixture some of the corrosive sublimate is washed out and a part of the methylal is replaced by the xylol. After remaining in this mixture a short time, the preparation is next placed in a considerable quantity of xylol. Here it should remain till all the methylal is replaced by xylol and the corrosive sublimate is washed out. As the last-named substance is only slightly soluble in xylol, the preparation should stay in this fluid some four or five days. At the end of this time it may be either mounted in xylol balsam and studied as a transparent object, or embedded in paraffin and cut in the usual

manner. The sections should be fixed to the slide with Schälli-baum's collodion and not with Mayer's albumen, which discharges the colour. Whole preparations or sections made in this way are serviceable for study for several weeks ; but after an interval of a month the finer details in them are likely to fade.

"The principal difficulties met with in employing this method are three :—A semi-crystalline condition of the precipitate, due, apparently, to over-action of the corrosive sublimate ; incomplete dehydration and imperfect removal of the corrosive sublimate. Remedies for these troubles easily suggest themselves.

"The essential steps in the method can be recapitulated as follows, the lengths of time given being those required for a satisfactory preparation of a ganglion in the ventral nerve-chain of the crayfish :—

1.—Cold, saturated, aqueous solution of corrosive sublimate for 10 minutes.

2.—Solution A :—Methylal, 5 ccm. ; corrosive sublimate, 1 gr. ; for fifteen minutes.

3.—Solution B :—Methylal, 1 vol. ; solution A, 1 vol. ; xylol, 2 vols. ; for ten minutes.

4.—Pure xylol in considerable quantities for 4 or 5 days.

5.—Mount preparation in xylol-balsam, or embed in paraffin and cut sections."

An Aqueous Solution of Hæmatoxylin which does not readily deteriorate.*—Prof. S. H. Gage—finding that aqueous solutions of hæmatoxylin soon begin to deposit a dark precipitate on the bottle and become filled with granules and mycelium growths—has devised the following solution, which, after a lapse of eight months, is as good as when first made :—Distilled water, 300 cc. ; Potash alum, 10 grams ; Chloral hydrate, 6 grams ; Hæmatoxylin crystals, 1/10th gram. Place the water in a porcelain dish, add the alum either in powder or small pieces, and boil for five minutes. When cool, add the chloral hydrate and the hæmatoxylin. It is advantageous to dissolve the hæmatoxylin in 5 to 10 cc. of absolute or 95 per cent. alcohol before adding to the alum solution.

The colour is quite light at first, but afterwards changes to a

* Read before the American Micros. Soc., 1892. *Micros. Bul.*, ix. (1892), pp. 36, 37.

dark purple. The solution may be concentrated by adding more hæmatoxylin. For dilution, alum, chloral, and distilled water answers best.

Method of Examining Blood, Bone - Marrow,*—Although Ehrlich's method preserves the characters of the red corpuscles and fixes the hæmaglobin, Dr. R. Muir, finding that the structure of the nuclei is not so well preserved by it, proposes the following method:—Films of blood are made on cover-glasses, as in Ehrlich's method, care being taken to avoid pressure on the films. They are then placed, before any drying can occur, film downwards, for about half-an-hour, on the surface of a saturated solution of corrosive sublimate, with $\frac{3}{4}$ per cent. sodium chloride added, preferably heated to a temperature of about 50° C. (though this latter is not essential).

They are then thoroughly washed in $\frac{3}{4}$ per cent. common salt solution, taken through successive strengths of alcohol, and then stained in the same way as sections. He also adds salt in the same proportion to the weaker strengths of alcohol. In the case of the bone-marrow, a little of the pulp is brought in contact with a cover-glass once or twice, so as to make a layer, but it ought not to be spread out—*e.g.*, by a glass rod, as thereby the cells become distorted. The cover-glass is then placed in the fixing solution. Spleen pulp, the juice of the lymphatic glands of tumours, etc., can be treated in the same way. The stains found most useful are Ehrlich's acid, hæmatoxylin with aurantia or with eosin, saffranin with aurantia, the triple stain of saffranin, hæmatoxylin and aurantia, and Biondi's triple stain. Dr. Muir states that he found the method very useful for photographic purposes.

Method of Preparing the Blood-Vessels of the Retina for Lantern Demonstration.†—Dr. J. Musgrove has been experimenting on the eye of the ox, and says that the eye should be obtained within a short time of death. In removing the eye, as much as possible of the fat and muscles of the orbit should be removed as well, and the vessels cut far back. The injection is made through the ophthalmic artery with an ordinary hand-syringe. Very good

* *Journ. Anat. and Physiology*, Vol. XXVI. (1892), pp. 393, 394.

† *Journ. Anat. and Physiology*, XXVI. (1892), pp. 244—253.

results are obtained with melted carmine-gelatine, but it is important to keep the eye in hot water for half-an-hour before the injection is made and after the nozzle has been inserted, in order that the gelatine may flow readily through the smallest vessels. It is better to arrest the "bleeding" points, especially the veins, while the injection is being made. He has generally found that it requires as much pressure as could be exerted with one hand in order to fill the vessels completely. The tension of the eye-ball and the state of the conjunctive vessels serve as a guide to the progress of the injection. Although carmine-gelatine gives very good results, it does not afford a complete view of the vessels, because the colouring matter in the capillaries is too small to produce any effect on the screen. To overcome this difficulty, I tried an injection mass composed of gelatine and a preparation of logwood, which gave excellent results. When the injection is complete, the eye must be cooled for a few hours in order to allow the gelatine to set. The next stage consists in removing the entire retina without tearing the membrane. This can best be done from the front. The cornea is removed by making a cut with scissors along its margin. Then the iris is removed in the same way, taking care to wash off any pigment from the iris which remains, since it is difficult to remove it from the retina if once it touches that membrane. The lens is next removed by cutting through the anterior part of the capsule, after which the vitreous, along with the capsule of the lens, may be withdrawn from the eye by pulling upon it with forceps, at the same time making pressure on the posterior part of the sclerotic with the other hand. After the removal of the vitreous, the retina will be found hanging down from the optic disc, and its attachment there is to be divided with a knife, sufficient room being allowed for the purpose by cutting away part of the sclerotic. The retina may be freed and floated out in water. With the aid of a soft camel-hair brush, the retina is now spread on the glass, and it is important that no hardening agent, such as alcohol, be used, since this has a tendency to cause unequal contraction of the gelatine. By carefully stretching the peripheral parts, and slightly crowding together the central portions, it will be found possible to adapt the whole retina to the flat surface of the glass. Dehydration is carried out by slowly drying for twelve

hours in an oven at a temperature below the melting point of gelatine. Should any air-bubbles have got between the retina and the glass, they must be removed by pressure with the camel-hair pencil before the specimen is dried. The retina is then clarified by allowing it to remain for two or three days under oil of cloves, until all opacity is removed. The clove-oil is drained off, and the retina covered with solution of balsam in benzole, and another thin lantern slide used as a cover-glass. Should it be desired to take a direct negative photograph of the vessels, this can easily be done, before the clove-oil is removed, by placing the silver paper directly in contact with the specimen and exposing it to the light. The clove-oil, which will have sunk into the paper, can be removed with methylated spirit, and the development proceeded with. Specimens prepared in the above manner are equally suitable for naked-eye and lantern purposes, and for microscopical examination if sufficiently thin glasses have been used.

Method of Killing Nematodes.—The following method for preventing Nematodes from curling while being killed is recommended by a writer in the November number of the *American Naturalist*, who has found it indispensable in fixing Nematodes and other worms :—

“The worm is placed in a few drops of water upon a large slide ; a second slide is placed over the worm and moved slowly to and fro. This movement causes the worm to straighten. As soon as the Nematode assumes the desired position, the fixing liquid is pipetted between the slides, the motion of the upper slide being continued until the worm is dead. By this method one can obtain a specimen which is perfectly straight and round. If the worm is delicate, too much pressure must not be used during the rolling process. Pressure may be avoided by pasting a piece of paper on the upper surface of the second slide and using that as an handle. As a killing liquid, the following solution is used :—Corrosive sublimate ; alcohol, 70 per cent. ; and a few drops of acetic acid, heated to 50° C., which passes through the cuticle very quickly.”

Half-an-Hour at the Microscope, With Mr. Tuffen West, F.L.S., F.R.M.S., etc.

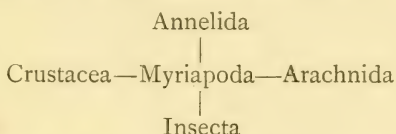
PLATES I., II., III., IV.

Stellate Hairs amongst the Sori of *Platyserium aleicorne* (Pl. I., Fig. 1) are both beautiful and interesting. The great difference between the lax, sparsely divided, greatly produced, hairs of the frond, and the compact, multifid hairs of the sori, is a significant fact.

Anchors, *Synapta inherens* (Pl. I., Fig. 2).—*Synapta* “is abundant, buried in mud-banks, at and a little above low-water mark, on the shores of Belfast and Strangford Loughs” (Wyville Thomson, *Q.M.J.*, 1862, p. 131). *A. de Quatrefages*’ so called *S. Dusernae* is a synonym; a closely allied form frequently met with on the English coast. *S. digitata* has been carefully described by Johannes Müller (Ueber *Synapta digitata*, Berlin, 1852). An elaborate memoir on the anchors and calcareous plates of the genus, by the late W. D. Herapath, appeared in the *Quarterly Journal of Micro. Science* some years back. Wyville Thomson gives, among other structural details, a most interesting account of the formation of these anchors and anchor plates, from the time when they first become to be visible to that of their completion.

This slide requires as its complement (failing living specimens) a portion of the skin in its dry state, and a section of the same, to show the projection of the anchors from the surface. There is an important note on the use of these anchors in a work called *Reisen im Archipel der Philippinen*, by C. Semper, of which a review will be found in the *Q.M.J.*, 1868, p. 163. “The anchors of the *Synapta* are by no means, as is often supposed, locomotive organs; when they have laid hold of any part, the animal cannot disengage itself without sacrificing them. They are, it is true, moveable in their basilar plate, but there are not any muscles destined to move them, and the will of the animal has no action on their movements. Besides, the body of the *Synapta* does not cling to the hand except when one touches it roughly. In reality, the *Synaptæ* crawl on stones and plants without hooking on to them, and in *Synapta Beselii* the anchors are lodged so deeply in the skin, that M. Semper believed in their complete absence until microscopic examination showed him the contrary.”

Centipede (Pl. I., Figs. 3-10).--Aberrant forms of life—"connecting links"—have always a special interest to the naturalist. Amongst such are the MYRIAPODA; these serve to connect Worms with Insects, and Crustacea with Arachnida. A diagram may express these relations in the clearest way, thus :—



As is well said by Van der Hoeven—"There is in the entire Animal kingdom a net, everywhere connected, and every attempt to arrange animals in a single ascending series must necessarily fail of success." (*Handbook of Zoology*, Vol. I., p. 289.)

Myriapods, in the first period of their life, have fewer rings, and only three pairs of feet, as with all true insects. As they grow new rings arise, and the number of feet is augmented. In this respect also they resemble ringed worms, whilst in the metamorphoses of Insects, the homologous parts, rings, segments, are not multiplied, but are developed unequally, or are united, to form the different divisions of the body in the perfect insect. The number, also, of simple eyes increases during the development of myriapods. The changes needed to convert an annelid into a myriapod, are elegantly set forth by Rymer Jones in his outlines. His remarks are too extended to be given here, but in brief the process consists in—1st, Conversion of external branchiæ into internal respiratory organs (tracheæ); 2nd, Strengthening the soft integumental organs by chitinous material, the simple setæ for progression to be modified into jointed limbs; and 3rd, Concentration of the nervous system (ed. 1861, p. 280).

Head of Gnat (Pl. II., Upper part).—The *Q.M.J.*, 1855, p. 97, has a remarkable paper by C. Johnston "On the auditory apparatus of the Mosquito"—a creature which differs but slightly in the main from our common English Gnat, *Culex pipiens*. The habits of the creature in a state of nature, experiments on the actions of sounds, and anatomical structure, all lead the author to consider that the greatly dilated basal joint in the male is the seat of the sense named. I must refer our members to the original paper for details,

only quoting the conclusion—"The position of the capsules strikes us as extremely favourable for the performance of the function which we assign to them ; besides which, these present themselves in the same light, the anatomical arrangements of the capsules, the disposition, and lodgment of the nerves, the fitness of the expanded whorls for receiving, and the jointed antennæ fixed by the immovable basal joint for transmitting vibrations, created by sonorous modulations. The intra-capsular fluid is impressed by the shock, the expanded nerve appreciates the effect of the sound, and the animal may judge of the *intensity*, or *distance*, of the source of sound, by the *quantity* of the impressions ; of the *pitch*, or *quality*, by the consonance of particular whorls of the stiff hairs, according to their length ; and the direction in which the modulations travel, by the manner in which they strike upon the *antennæ*, or may be made to meet either *antenna*, in consequence of an opposite movement of that part. That the male should be endued with superior acuteness of the sense of hearing appears from the fact, that he must seek the female for sexual union, either in the dim twilight or in the dark night, when nothing, save her sharp humming noise, can serve him as a guide. The necessity for an equal perfection of hearing does not exist in the female ; and, accordingly, we find that the organs of the one attain to a development which the others never reach. In these views we believe ourselves to be borne out by direct experiment, in connection with which we may allude to the greater difficulty of catching the male mosquito. In the course of our observations, we have arrived at the conclusion, that the *antennæ* serve, to a considerable extent, as organs of *touch* in the *female* ; for the palpi are extremely short, while the antennæ are very moveable, and nearly equal to the proboscis in length. In the male, however, the length and perfect development of the palpi would lead us to look for the seat of the tactile sense elsewhere ; and, in fact, we find the two apical antennal joints to be long, moveable, and comparatively free from hairs ; and the limited motion of the remaining joints very much more limited." (*loc. cit.*, pp. 101—102.)

Anatomy of Drone-fly.—A thoughtfully prepared dissection of a kind much to be desired in our boxes. The importance, in

choosing the subjects, of taking *types of form*, must be carefully borne in mind. So long as they are good "types," the commoner the better. It is desirable to either accompany or precede the dissections by an example of the object in its entire state ; in some cases a good coloured drawing may be the only means of practically doing this. Some good work should, in all cases, be taken as a guide, and the assistance of a friend with knowledge and experience will be invaluable, especially at the outset. The contributor speaks of THE spiracle as if there were but one ; that usually sold under the name, and which he doubtless intended, is the second or meta-thoracic, situate at the base of the balancers. Besides this, there is one on the pro-thorax, a little above the attachment of the first limb, and in addition, one on each side of the five abdominal segments, besides one to nearly each segment of the overpositor. So that, taking the Blow-fly as the type of the Diptera, there are ten spiracles on each side in the male, and eight in the female (*Lowne on the Blow-fly*). The explanation of this discrepancy doubtless has reference to the greater activity of the male in pursuit of the opposite sex, and consequent need of more highly developed respiratory organs ; the females being often more sluggish in their habit.

Gizzard of the Green Weevil (Pl. II., Figs. 1-3).—This slide differs from others which I have seen, only in minor particulars. The "teeth," which have their points directed towards the mouth, are seated on elevated ridges ; in all the examples from weevils which I have seen, they are thin chitinous plates, curiously resembling the scales of Lepidoptera, both alike having longitudinal ridges running out at their free extremities into spines, with shorter transverse ridges. The more or less horny membrane on which they are seated (the "gizzard bag," it might be called), is surrounded by an inter-lacement of muscular fibres, whereby its effective action is produced. The gizzard of the Cockroach offers a favourable subject of examination of the muscular structures ; they are the parts which the contributor of this slide calls "the skin," and which it is generally preferable to leave *in situ*, when not so thick as to interfere with the examination.

Section of Small Intestine of Mouse (Pl. III., Upper portion).

In this preparation some interesting points in the blood-supply are well shown. The part is that which succeeds to the stomach; it is in it that the absorption of nutrient materials, from food received, principally occurs. To increase the absorptive surface, it is raised into innumerable finger-like processes called "*villi*." Certain smaller vessels may be seen in the line of axis of the villi, which are the Arteries; there is also one vessel of larger calibre by which the blood is carried away—the Veins. Proceeding across the villi, and constituting a network over and a little within its surface, are finer vessels, the capillaries (*Capillus*, a hair). On reflection it will become evident how admirably such a sponge-like arrangement is adapted to the purposes of an organ, alternately turgid through the stimulus of present food, and flaccid during intervals of abstinence. The surface of the villi is covered with conical epithelium cells, whose office is to absorb the liquid aliment, and to pass it on to channels excavated in the villi, the lacteals (*lac*, *lactis*, milk), from the milky appearance of their contents. The lacteals unite, and receive the name of lymphatics, and the contained fluid is eventually poured into the great veins of the neck on the left side, just above the heart. In the lungs a wonderful change takes place, the "chyme" becoming changed into fully formed blood.

Cheyletus eruditus (Pl. III., Figs. 1-4).—R. Beck was not the first to discover this insect. It will be found mentioned in an *Enumeration of the Insects of Austria*, by Schrank, published towards the close of the last century (1792, *circa*), and afterwards by Latreille, in the *Natural History of Crustacea and Insects* (1806—9). R. Beck found it independently in 1866, and though he did not know what he had got, to him belongs the merit of accurately observing its life-history, as well as carefully describing and figuring it. McIntire's paper in *Science Gossip* partakes somewhat of the sensational, and his account is rather confused in parts. The figure at its commencement is thought by M. C. Cooke to represent, probably, *C. verrutissimus*, Koch. As to oviposition, I doubt the weaving of threads around the eggs to retain them in their places, either from the mouth or elsewhere, and think there must be an error of observation. By R. Beck's description the eggs are attached by a short thread of condensed mucus to the

bodies on which they are placed, as in the Lace-wing fly, the Lady-bird, and *Alcyrodes Cheledonii*. Trustworthy observations cannot be made upon the fact, except in living specimens, R. Beck says—"The last joint of each tarsus is furnished at its extremity with two hooks, and two longitudinal and parallel rows of delicate tenent hairs; by the aid of these the *Acarus* walks with some little hesitation in an inverted position upon glass." His figure represents a narrowly linear, undivided pulvillus, with eight tenent hairs on either side. S. J. M. says nothing of the remarkable brooding of the female over the eggs, so graphically described by R. Beck.

I once found a *Cheyletus* (species undetermined) running over a dead chicken; and in one of the insect cabinets in the vaults at the British Museum I was shown a moth with several acari on its wings. From a passage (I think in Kirby and Spence) it seems to be not unlikely these may have been *Cheyleti*. This was, however, many years ago, and they were not examined with the microscope.

T. WEST.

Selected Notes from the Note-Books of the Postal Microscopical Society.

Mites, To Mount.—Mites, because of their transparency, look better if stained. I have mounted some stained specimens from humble-bees, and every part is thoroughly distinct.

H. M. J. UNDERHILL.

Cheyletus eruditus (Pl. III., Figs. 1-5).—I imagine that the parts which I call falces (*f.*, Fig. 2) are analogous to the falces of spiders. I would call attention to the two beautifully delicate combs on each falx. At first I thought there were three—viz., two of the larger sort and one small; but more careful examination showed me that I was deceived by two extremely slender claws, which by refraction of light through the comb appeared to have teeth also when the objective was slightly out of focus. These combs are moved by three or four muscles which arise at the outer edge of the basal joint of the falx (Fig. 4). The muscles which move the chief claw have their origin further down.

In the mouth proper I fancy I can detect two exceedingly minute mandibles, one on each side of the rostrum, *r.* The rostrum is hollow and retractile. It is moved by *exsertor* and

retractor muscles (*e.r.* and *r.r.*, Fig. 2). The structure of this organ reminds one slightly of the mouth of a sheep-tick. The feet are curious and possess a pair of claws (a cheese-mite has only one), a pad, and two or more tenent hairs. Cheyleti are very scantily furnished with hairs. It will be noticed that those on the last joint of the fore-feet are much longer than on the last joints of the other feet. Cheyleti use their fore-feet as feelers, having no eyes, just in the same way as certain gnats use theirs ; consequently, the hairs are longer to render them more sensitive.

In *Science Gossip*. 1869, p. 5, some account of the Cheyleti and their habits may be found. The following is a digest of that paper :—Cheyleti feed on cheese-mites and other acari, which they generally seize by the leg. They obtain nutriment by suction. They are to be found on rotten wood, spiders' webs, etc., in old cellars. They are active. They were first thought to be hermaprodite (but, as the contributor to the slide under observation says, it contains two males and one female, this statement is doubtless false). At any rate, several generations of females produce young without the intervention of the male, as Aphides do. The eggs are laid in corners and kept from rolling about by being secured by threads crossing in various directions. These threads appear to be spun by the animal from the mouth (I doubt this, but cannot detect any spinnerets). Cheyleti were first found by R. Beck.

H. M. J. UNDERHILL.

Head of Gnat.—I find the best way to mount these delicate antennæ is, after stupefying the gnat with chloroform, to sever the head with a pair of fine-pointed scissors, and let it fall direct into oil of cloves. After remaining there a few days or a week, I float it at once on to a slide and mount in Canada balsam. A. A.

Dolichopus (Pl. IV., Upper portion).—I have given drawings of a foot and an antenna of an allied species, *Dolichopus longicornis*, to show some peculiarities of the genus. If the foot of the Dolichopus on the slide be examined, it will be observed that the terminal joint of the tarsus is considerably thicker than any of the others. Nevertheless, the difference in size is quite small when compared with that of the tarsus of *D. longicornis* (see Figs. A and B). There is another species, *D. discifer*, which has tarsi exactly intermediate in form to these two, even to the form of the last joint but one.

This enlargement of the last joint, which amounts almost to grotesqueness in *D. longicornis*, extends chiefly in one direction—*i.e.*, in the figure the greater diameter is shown ; if the foot were seen from above, the last joint would appear but little broader than the others.

In some species of *Dolichopus* the other feet have peculiarities

also, but in the slide before us the other feet are of the ordinary form. In this genus the antennæ are very variable. *C.*, Pl. IV., shows the antenna of *Dolichopus longicornis*. The antennæ are peculiar in structure; but I think with the aid of the diagrams *D* and *E* they may be understood. The second joint, *D*, has a thumb-like process projecting from one side; this process is received into the interior of the helmet-like third joint, *E*, and is articulated to the bottom of it, in the same way as a clapper is articulated (so to speak) to the bottom of a bell.

F. J. ALLEN.

Wings of Insects are always spoilt by caustic potash. To save them entire, they should be cut off from the insect before it is treated with potash, and placed in position on the slide at the time of mounting.

F. J. A.

Haltere of Fly.—These organs, with some few exceptions, are even more delicate than wings and are totally ruined by potash. They are very beautiful objects, but their chief interest lies in their being the probable seat of the *sense of hearing*. In his "Anatomy of the Blow-Fly" Mr. Lowne goes deeply into the subject. His conclusions are that the antennæ are *not* organs of hearing, but of smelling; and that if insects hear at all they hear with their wings.

In the wings of most insects, on one of the larger nervures near the base, may be seen a group of little spherical bodies embedded in the substance of the wing. The theory connected with these is that they are *otoconia* or ear-stones, floating in fluid, and connected with auditory nerves, and that the sonorous vibrations of the air are communicated to them by the membrane of the wing, which acts as a tympanic membrane. It is believed that in the Diptera one pair of wings is modified into halteres for the special purpose of hearing, for although *otoconia* exist in the wings proper, they are much more developed in the halteres; moreover, the haltere seems to be eminently adapted for receiving impressions of sound.

The haltere consists of a delicate membrane enclosing some kind of fluid. It is strengthened by two nervures—one at the anterior, the other at the posterior border, which are relics of the nervures in the wings of insects. In the interior are a number of oval, cell-like bodies with nuclei, whose functions I do not at all know.

At the base of the haltere are situated two groups of *otoconia*: one on the posterior nervure, the other on the soft portion of the haltere contiguous to the body. The latter group are irregularly arranged, and do not appear in my drawings on the plate; but the former are arranged in rows across the nervures, and are shown at Fig. *F*, and much more magnified in Fig. *G*, Pl. IV.

When very highly magnified, they appear, as shown at Fig. *G*, as rows of little spheres, with irregular dumb-bell-like objects between them, a space lying between two rows, with a row of bristles on each space. I do not understand the "dumb-bells"; they are, perhaps, not solid, but merely illusory appearances, caused by light refracted and reflected from the otoconia.

Some idea of the smallness of the otoconia may be gathered from the fact that Fig. *G* is magnified 630 diameters. In the haltere on the slide under discussion the otoconia cannot be seen, but their *sheaths* are plainly visible when the haltere is torn near the base. Space and time will not permit me to fully discuss the function of the so-called otoconia; but I recommend all who can to read what Mr. B. T. Lowne says on the subject in his valuable work on the *Blow-Fly*.

I have omitted to mention that at the end of most halteres (and probably all) there is a flat, oval, more transparent portion which seems peculiarly like the tympanic membrane in the ears of mammalia.

The insects from which the drawings of the haltere and otoconia were made are two of the best diptera for studying these organs by. The common Blue-Bottle, however, shows these organs as well as most insects.

FRANK J. ALLEN.

Flea.—The muscles of this insect may be well shown by soaking a freshly-killed flea successively in ether, water, weak spirit, absolute alcohol, and oil of cloves, and finally mount in Canada balsam without pressure.

FRANK J. ALLEN.

Centipede.—Mr. West's careful drawings of the Centipede (Pl. I., Figs. 3—10) are very interesting, and bears upon a subject respecting which I should like to know more. In the first place, is it to be regarded as an undoubted fact that the segments or rings of insects are not multiplied as Mr. West states? Audouin, many years ago, in a paper published in the *Annales des Sciences Naturelles*, endeavoured to show that each segment of the thorax of insects was normally composed of four sub-segments. Now, I have not had an opportunity of seeing this paper, and do not know what reasons were adduced for such a conclusion; but certain phenomena connected with the thoracic appendages have lately attracted much of my attention, leading forcibly to a similar result. Audouin's views seem to have dropped completely out of sight, for I nowhere find the number of segments in insects put down at more than seventeen; indeed, in all but recent treatises, thirteen is quoted as the normal number, and it was only after forming my own opinion on the subject that I became aware of its coincidence with that of Audouin.

I cannot here enter into the discussion of this subject. Suffice

it to say that the reasons I have for so thinking seems to me to apply to the abdominal segments also. It will, of course, be seen that if this is the case, the typical number, seventeen, is greatly understated. It would seem, further, to come back to my starting point, that from the marked character of the segmentation hitherto recognised, such sub-segments, if they exist, must have been produced by duplicative subdivision of the former; thus, roughly and diagrammatically, for example (see Diagram, Fig. 1, Pl. IV.), as regards the thoracic segments. I hope I shall not be thought overbold in this speculation, seeing that I am held in countenance to some extent by no mean authority. I shall endeavour to direct my attention further thereto.

I should much like to see Rymer Jones's *Outlines*, alluded to by Mr. West. I gather that the external branchiæ of Annelids are herein regarded as the homologues of the tracheæ of the Myriapoda and Insecta, thus confirming a hint thrown out by Mr. Lowne that these latter are true appendages developed inwardly. It will be observed from Mr. West's drawing (Pl. I., Fig. 4) that the spiracles occur opposite the legs towards the dorsum immediately beneath the lateral edges of the dorsal plates, just the very position where they should occur as superior dorsal appendages.

ARTHUR HAMMOND.

Anatomy of Drone-Fly (Pl. IV., Lower portion).—The ovipositor presents similar features to that of the Blow-fly, but the male organs are different. The drawings are copies of some of my own made some time ago. The eighth dorsal abdominal plate appears by a curious process of torsion on the ventral aspect. The minute chitinous spaces on the membranous part of the integument, marked *5d.*, *6d.*, and *7d.*, are the fifth, sixth, and seventh dorsal plates. This appears from the occurrence of spiracles between them and the corresponding larger ventral plates, marked *5v.*, *6v.*, and *7v.*

The bilateral symmetry usually observable in insect structure appears here to be wholly wanting, as, indeed, it frequently is in these parts. The plates seem to be twisted over, so that on the dorsal aspect we see a little piece of the sixth ventral plate, a larger piece of the seventh, and nearly the whole of the eighth; while on the ventral aspect the reverse takes place: we see nearly the whole of the sixth, less of the seventh, and only a little piece of the eighth. In the same way, the dorsal plates represented by the eighth (for the fifth, sixth, and seventh are mere rudiments) are presented on the ventral aspect. This may seem strange, but an examination of the parts in their natural condition will show that it is most difficult to determine what does follow the eighth ventral plate from observation alone. I have therefore followed

the lead indicated by the preceding segment, and the analogy presented by the Blow-fly where the anus opens between the dorsal valves or appendages of the eighth and ninth segments.

The relations of the succeeding parts, including the male generative organ, are very obscure. I have indicated my opinion about them in the lettering of the figures, but feel it is little more than conjecture.

A. HAMMOND.

Halteres of Diptera.—If the theory be true that in the Diptera one pair of wings is modified into Halteres for the special purpose of hearing, that sense ought to be considerably more acute in the Diptera than in other insects. Is such the case? J. H. GREEN.

Haltere of Blow-Fly.—I know that a certain mounter circulates the Halteres as "Buzzing Organs"; whatever they may be, they are certainly not that.

The Halteres of the Diptera take the place of the second pair of wings in four-winged insects, and they are, in fact, transformed wings. They are connected to the upper posterior sides of the thorax by a joint, and are raised and lowered by special muscles.

Their use seems to be to enable the insect to direct its flight. They act by displacing the centre of gravity. Experiment shows that, deprived of them, the insect, on attempting to fly, falls at once; but if a small weight be attached to the abdomen so as to bring the centre of gravity behind the axis of suspension, the power of directing the flight is restored (*Comptus Rendus*, 1879, p. 89).

At the base of the haltère are four sets of special organs: two sets on the upper and two on the lower surface. Each set consists of a series of curved ridges, under each of which is a row of hemispherical or oval vesicles, numbering nearly a thousand in all. Each ridge is divided from its neighbour by a row of curved hairs.

The vesicles are described by Dr. Hicks as openings in the chitinous integuments, closed by a thin, cuticular membrane, whereby a longer or a shorter tube is formed.

Dr. Lowne denies the assertion that the vesicles are openings in the integument, and says they are lenticular corpuscles of high refractive power. He considers the corpuscles to be otoconia and the organs to be those of hearing; and as a parallel instance mentions that the auditory organs of some orthoptera are developed on their anterior femora.

Dr. Hicks considers them to be organs of *smell*, their position (close to the posterior thoracic spiracles, like the position of the olfactory organs in the nostrils of vertebrata) being particularly suitable for detecting odoriferous particles in the streams of air inspired into the body. Which of the doctors is right?

The sense of which they are the organs is one of great import-

ance to the insect, for the nerve supplying it is, with the exception of the optic nerve, the largest in the insect's body. On entering the haltere it splits up into a multitude of filaments, one passing to each vesicle, while the main branch proceeds along the shaft of the haltere and ends in a loop in the globe. T. C. WATSON.

Halteres.—I find it stated that a Crane-fly continued to buzz after being deprived of its halteres, so that they cannot have much to do with that performance. The same authority states that a Crane-fly deprived of one or both its halteres or winglets could not fly at all, and he concludes that they must be used as air-holders. Derham, on the other hand, states that Diptera, when deprived of one of their halteres, flew one-sided, and he thinks they must be to steady the flight. Probably they are compound organs and used for both purposes—viz., for smell and as poisers.

E. S. ANGOVE.

Halteres.—A quotation from Hurley's *Manual of the Invertebrata* will probably show that the function of the halteres is not of such importance as the above writers appear to suppose. On p. 439 it is stated :—"In many winged insects both pairs of wings are developed, and take equal shares in flight. In the Diptera the posterior wings are represented only by short processes of the halteres. In the Strepsiptera, on the other hand, it is the anterior pair of wings which abort. In all orders of winged insects, individual cases of complete abortion of the wings occur, either in the female alone or in both sexes.

R. L. HUDSON.

Halteres.—Perhaps the most convincing proof that the halteres of flies are modified wings is to be found in the fact that in the pupa of many of these insects these organs are to be seen nascent in what must be called true wing-cases, precisely similar to those of the fore-wings in every respect except that of size. Thus, in the Crane-fly, Pl. IV., Fig. *H* shows the fore-wing case of the pupa with the nascent wing inside. Fig. *I* is the hind-wing case, with the haltere inside. It is impossible to doubt with such evidence that the organs are homologous. The same thing may be distinctly recognised in the pupa of the gnat, and even in the larva when arrived at maturity.

A. HAMMOND.

Mica is a mineral occurring in metamorphic rocks ; it consists of bright, shining plates, which can be split up into very thin laminae. With polarised light it appears of a variety of lines, the colour depending on the degree of thinness of the lamina.

H. F. PARSONS.

Granite and Syenite are both rocks of igneous origin, formed, under great heat and pressure, from masses of erupted matter

thrust in among the substance of the older strata, or from the alteration of the pre-existing strata themselves.

Granite consists of three minerals: quartz, mica, and felspar. Syenite is of similar composition, except that the mica is replaced by a greenish-black crystalline mineral called horne-blende, seen in the section as greenish crystals. Felspar occurs in crystals which have a somewhat elongated form and ragged outline, due to their being crossed in two directions by cleavage planes. One set of cleavage planes (the most conspicuous) is longitudinal; the other transverse. There are two varieties of felspar: orthoclase, in which the planes are at right angles to each other, and plagioclase, in which they lie obliquely.

H. F. PARSONS.

Section *Stenocarpus Cunninghami*.—Stained by Dr. Beattie's method. The aniline colours stain all too quickly; yet the details of the section *continue* to come out much more plainly than in others which have never been stained.

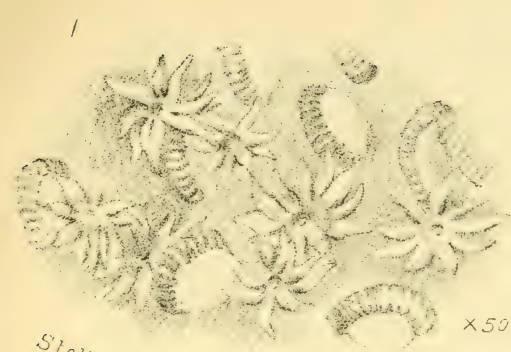
The genus *Stenocarpus* is one of the *Proteaceæ*, an order of perigynous exogens, comprising some forty-five genera, nearly all of them natives of the Cape of Good Hope or Australia—shrubs or trees with hard dry leaves, often showy flowers, and often differing very much in external appearance; hence the name of the order. The present species is a lofty tree, with handsome orange flowers in terminal umbels, and is found in Queensland and other semi-tropical parts of Australia. The section is made from a small twig of a tree growing in an English conservatory, and shows under the microscope small sphæraphides, and, also, both in the centre and round the edge, numerous angular semi-opaque nodules of very various sizes. Are these resinous or siliceous concretions? I do not remember to have seen any such in *wood* sections before, though in many of the seeds of commerce they are not uncommon. It would be useful to know if the wood furnishes any resinous or other useful products.

J. H. GREEN.

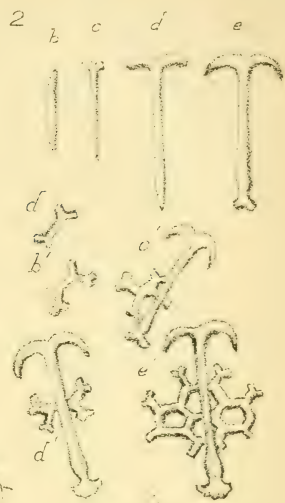
EXPLANATION OF PLATES I., II., III., IV.

PLATE I.

- Fig. 1.—Represents some of the Sporangia of *Platyserium alicorne*, with stellate hairs intermixed.
- „ 2.—Shows the various stages in forming Anchors and Anchor-plates of *Synapta inherens*, after Wyville Thomson. The first stage consists of minute calcareous granules (*a*); the anchors precede the plates; a slender rod is the first indication (*b*). By accretion this enlarges somewhat, and grows out laterally on either side of one end (*c*); by continuance of the process through (*d*) the perfect state is nearly arrived at. In the meantime, underneath the anchor—that is, nearer the interior of the



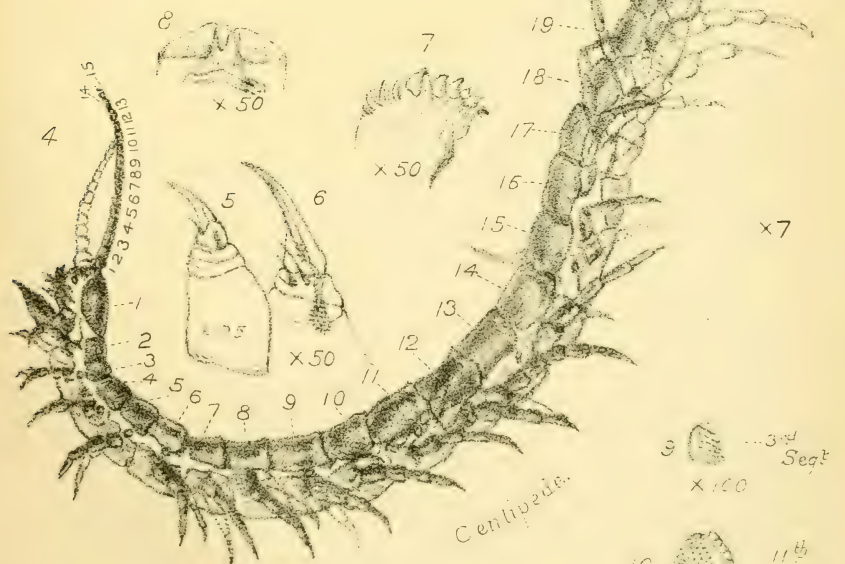
Stellate hairs of *Platycerium*.



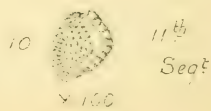
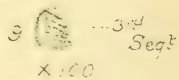
Spicules of *Symapta*.

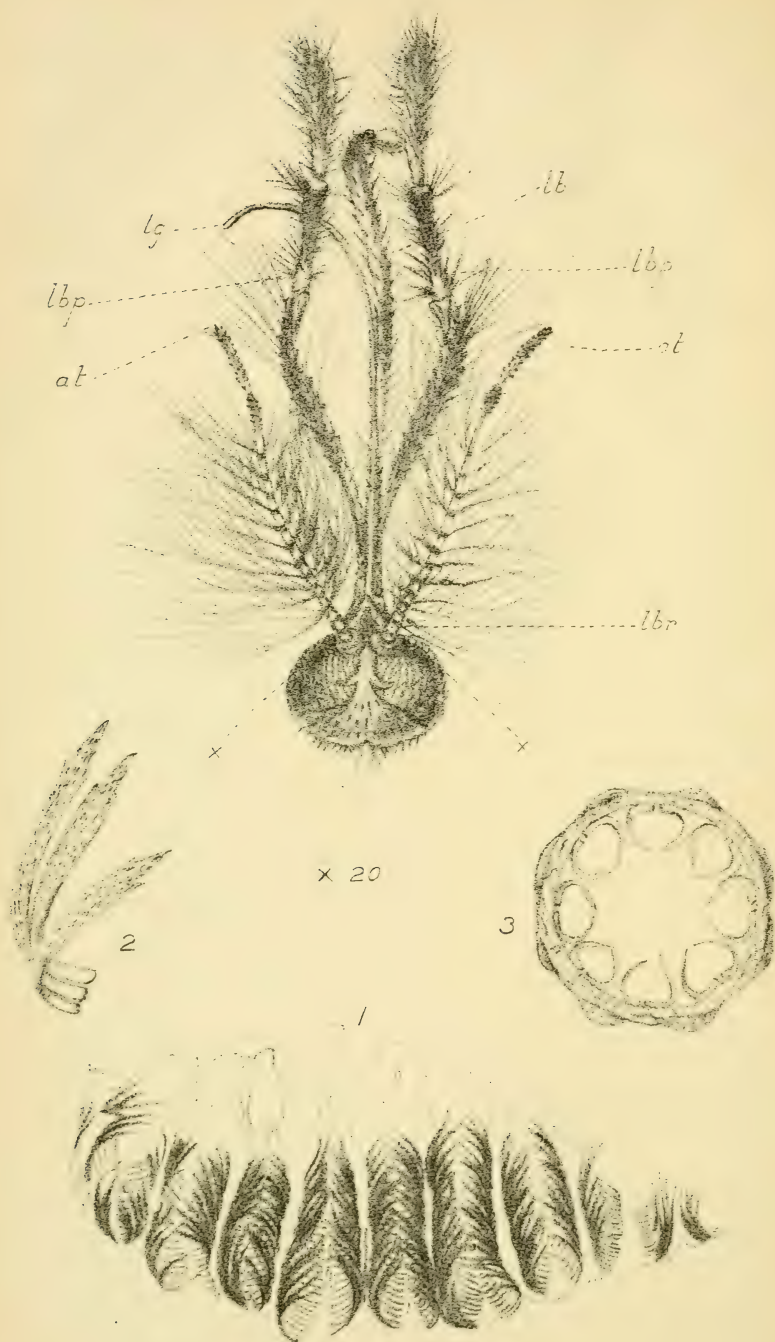


Mandible of Centipede.



Centipede.





body—a corresponding process of accretion has been taking place, represented by figures *a'*, *b'*, *c'*, *d'*, *e'*, whereby a calcareous network with hexagonal areolæ is formed. By degrees this becomes more shaped, and as a finishing stroke the edges of the network entirely become denticulate, whereby the power of holding to the skin, and so affording a firm resting-bed for the anchor, must be very greatly increased.

- Fig. 3.—Mandible of *Lithobius forficatus*, the common brown Centipede, $\times 50$, to show the poison-gland, with its investment of spirally arranged, unstriped muscular fibre, the long slender duct, and the slit near the extremity of the fang, where the poison finds exit through a very minute opening.
- „ 4.—*Lithobius forficatus*, as seen with a very low power ($\times 7$). Counting the head as one, it will be found there are twenty-two segments, fifteen joints in the antennæ, and twenty pairs of limbs; thirteen pairs of spiracles, belonging to the 3rd, 4th, 5th, 8th, 9th, 11th, 13th, 17th, 19th, and 21st segments respectively. These vary in size, the 3rd, 5th, and 8th being very small, the 18th small, and the remainder of a larger size. *g.o.* shows the genital orifices.
- „ 9 on Plate represents the small spiracle of the 3rd segment, and Fig. 11 one of the larger size from the 11th segment, which is a good average of the large ones, both $\times 100$.
- „ 5.—The mandible of the left side, seen from below, $\times 50$.
- „ 6.—The same, more enlarged, showing the sieve-like openings of the receptaculum veneris and the duct.
- „ 7.—Right mandible from below. At the inner angle is a brush formed of three or four tufts of hairs; the teeth of the mandible are seen to be themselves denticulate.
- „ 8.—Labrum and portion of labium. Drawn by Tuffen West.

PLATE II.

Upper Portion.

Head of Male Gnat, showing, *at. at.*, antennæ of fifteen joints; *x x*, the enlarged basal joints. These latter, and the two terminal ones, are free from whorls of hair. *lbr.*, labrum; *lb.*, labium; *lb.p.*, labial palpi; *lg.*, lingua. $\times 20$.

Lower Portion.

Fig. 1.—Gizzard of Weevil. The entire specimen, as seen with a moderate power. $\times 90$.

- „ 2.—Three of the scale-like teeth, highly magnified. $\times 400$.
- „ 3.—Diagrammatic section of the entire Gizzard, in a distended state. It will be easy to ascertain the nature of the food by examining the contents. In *Hyllobius abietis*, the largest English weevil, portions of the bark of the Scotch fir were contained in a gizzard I dissected. In the present instance, I have no doubt the softer portions of leaves of nettle, hazel, and perhaps other plants would be found.

Drawn by Tuffen West.

PLATE III.

Upper Portion.

Fig. 1.—Transverse section through the small intestine of mouse.
× 20.

„ 2.—A single villus. × 100.

„ 3.—Diagram of part of the extremity of a villus, showing, *c. e.*, conical epithelium; *a.*, artery; *v.*, vein; *c.*, capillaries; *l.*, lacteal. Drawn by Tuffen West.

Lower Portion.

Fig. 1.—Egg of *Cheyletus*, 2/3rd in. objective, and A eyepiece. From *Science Gossip*.

„ 2.—Head of *Cheyletus eruditus*, ♀, showing muscles, etc. *f.*, falces; *r.*, rostrum; *r. r.*, retractor muscles of rostrum; *e. r.*, extractor muscles of rostrum. × 143.

„ 3.—Foot of same. × 425.

„ 4.—One falx of same, × 250, from a slide in the possession of the writer, showing muscles and comb-like falces more plainly than in Fig. 2. Drawn by H. M. J. Underhill.

PLATE IV.

Upper Portion.

A.—First tarsus of *Dolichopus longicornis*, × 25.

B.—First tarsus of another species of *Dolichopus*, × 25.

C.—Antenna of *Dolichopus longicornis*, × 25.

D, E.—Diagrams of second and third joints of the same.

F.—Haltere of *Dioctria rufipes*, a dipterous insect, showing otoconia at *x*.

G.—A few otoconia from haltere of *Tachino virgo*, a dipterous insect, × 630. Drawn by F. J. Allen.

H.—Fore-wing-case in pupa of Crane Fly, showing undeveloped wing.

I.—Hind-wing case of same, containing haltere.

Lower Portion.

Fig. 1.—Diagrammatic sketch of the duplicative sub-division of the thoracic segments.

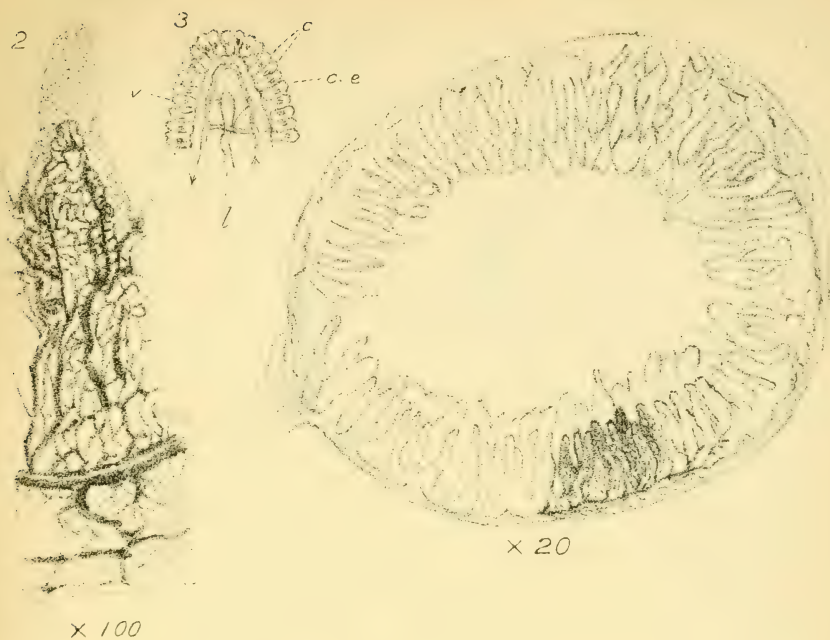
„ 2.—Sexual segments of *Eristalis tenax* (male). The several plates are indicated by numerals, followed by the letters *v.* or *d.*, to indicate whether ventral or dorsal; thus, 8*v.* is the eighth ventral abdominal plate, dorsal aspect. The figure shows the increasing extent of exposure of the sixth, seventh, and eighth ventral plates towards the dorsum.

„ 3.—The same, ventral aspect, showing diminishing exposure towards the venter of the same segments, and the eighth dorsal plate wholly turned towards the centre.

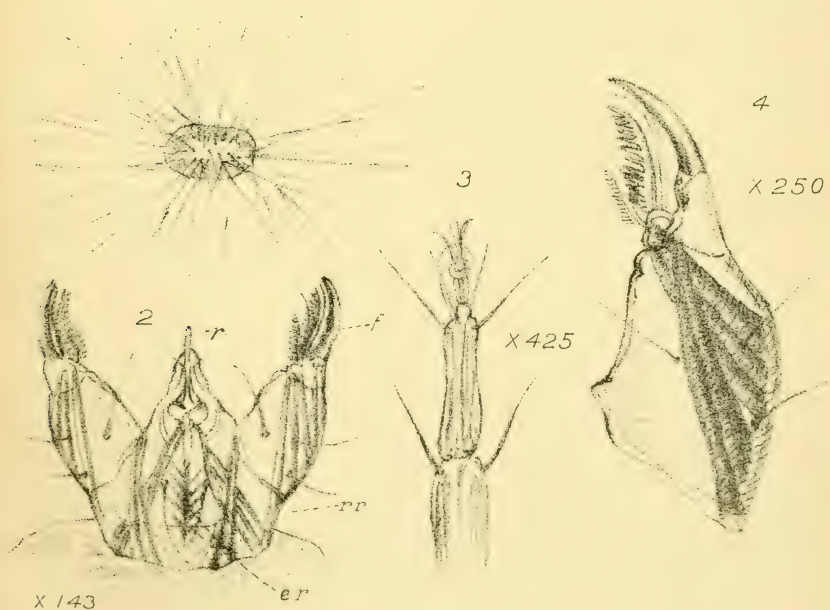
„ 4.—The male generative organ and surrounding parts: front view.

„ 5.—Ditto, side view, marked *p.* in Figs. 2 and 4.

„ 6.—Same segments from another slide, similarly lettered for comparison. Drawn by A. Hammond.

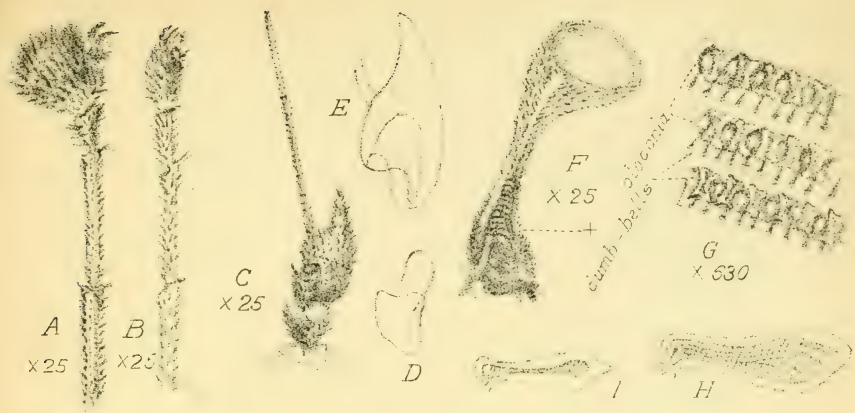


Small Intestine of Mouse.



Cheyletus eruditus





A. First Tarsus of *Dolichopus longicornis*

B. First Tarsus of Dolichopus.

C. Antenna of *Dolichopus longicornis*

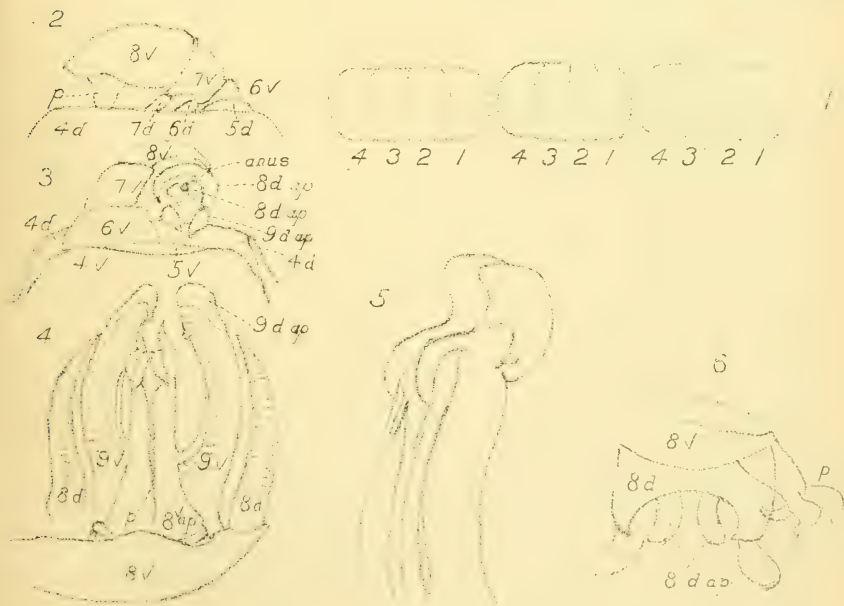
D. Diagram of 2nd joint of same.

E " " 3rd " " "

F. Haltere of *Dioctria rufipes* + *otoconia*.

G. Otoconia from Haltere of Tachina Virgo.

H. Fore wing-case of Crane-fly (pupa) I. Hind wing-case with Haltere.



The Microbe of Malarial Fever.

DR. Hoisholt, of Stockholm, U.S., has read a paper at the Medical Society of the State of California on the "*Plasmodium Malariae*." After mentioning the principal ideas brought forward as to the nature of the disease from the time of Hippocrates to the middle of the present century, the author alluded briefly to the most noted germ theories, and the character of the different microbes, claimed, by various investigators, to be the cause of malaria. 1st, Salisbury's unicellular alga, *palmella gemiasma*; 2nd, Lanzi's germ-ferment, identical with *bacteridium brunneum*; 3rd, Eklund's fungus, *limnophysalis hyalina*; 4th, Kleeb's and Tomasi-Crudeli's *bacillus malariae*; and 5th, Laveran's *oscillaria malariae*, now known as *plasmodium malariae*.

Since this French observer first published his fundamental researches (1881), many eminent investigators have corroborated his discovery, and have contributed largely to our knowledge of the parasitic malaria, having ascertained that it attacks the red blood corpuscles, lives and grows with them, and finally brings about their disintegration. It can be observed as follows:—After taking the proper precautions in removing the blood from the tip of the finger, it is fixed on cover glasses, and heated at a temperature of 105° to 110° C. (220° to 230° F.), for about half-an-hour. The cover-glasses are left for twenty-four hours in a neutral solution, consisting of equal parts of a $\frac{1}{2}$ per cent. aqueous solution of eosin, and a saturated aqueous solution of methelyne blue, diluted one half with distilled water. This is Romanowsky's colour test. He examined the blood in this manner in fifty cases of different diseases, not malarial, and in health, without being able to detect anything of the microbe in question.

—*Mon. Mag. Pharmacy.*

Notes.

WE are glad to note that the *Conversazione* of the Royal Microscopical Society, held at St. James's on Nov. 30th, passed off so successfully. Of late years the interest in the Society's exhibitions fell off considerably, owing principally to want of space and also to the fact that ladies were not admitted. Now that the "Royal" has again interested itself in the success of its *Conversazione*, microscopists will look forward to it as being the principal microscopic, or rather macroscopic, event of the year.

It is exhibitions of this kind that give an impetus to microscopy by interesting persons in the study of the wonders of the *semi-invisible*.

One of the principal exhibits at the *Conversazione* was that of the Marine Biological Association, showing the different stages in the development of food fishes, and also an ingenious apparatus by which colour is made to assert itself on the underside of flat fish, and which will probably give a clue to the piebald appearance of many fish which has puzzled investigators so long. Considering the almost national importance of the work done by the M.B.A., it is to be regretted that it is not sufficiently known to be appreciated at its proper value. To those of our readers who are interested in the study of marine life, we would call attention to the advantages of the association. The M.B.A. issue a price list of Marine Algæ, and of Zoological specimens (ranging from Protozoa to Fishes), which are supplied at an extremely cheap rate, and we strongly advise all who would continue their investigations in these subjects to communicate with the Association at their Laboratory at Plymouth. By doing this they will derive the double satisfaction of getting subjects for investigation, and of contributing, in no slight degree, to the success of the Association.

Speaking of the enormous variety of insect life, Dr. C. V. Riley, in the *Bulletin* of the U.S. National Museum, No. 39, says: "The omnipresence of insects is known and felt by all; yet few have any accurate idea of the actual numbers existing, so some figures will not prove uninteresting in this connection. . . . Linnæus knew nearly 3,000 species, of which more than 2,000 were European and over 800 exotic. The estimate of Dr. John Day, in 1853, of the number of species on the globe, was 250,000. Dr. Sharpe's estimate, 30 years later, was between 500,000 and 1,000,000. Sharp's and Walsingham's estimate in 1889 reached nearly 2,000,000, and the average number of insects annually described since the publication of the *Zoological Record*, deducting 8 per cent. for synonyms, is 6,500 species. I think the estimate of 2,000,000 species in the world is extremely low, and if we take into consideration the fact that species have been best worked up in the more temperate portions of the globe, and that in the more tropical portions a vast number of species still remain to be characterised and named, and if we take further into consideration the fact that many portions of the globe are yet unexplored entomologically, that in the best worked-up regions by far the larger portion of the Micro-Hymenoptera and Micro-Diptera remain absolutely undescribed in our collections, and have been but very partially collected, it will be safe to estimate that not

one-fifth of the species extant have yet been characterised or enumerated. In this view of the case the species in our collections, whether described or undescribed, do not represent perhaps more than one-fifth of the whole. In other words, to say that there are 10,000,000 species of insects in the world, would be, in my judgment, a moderate estimate."

The American Microscopical Society offer the following prizes for the encouragement of microscopical research :—

Two prizes of 50 dols. each for the best papers, and two prizes of 25 dols. each for the next best, which give results of an original investigation on animal and plant life respectively, made with the microscope. The papers are not to be less than 3,000 words in length and the methods by which the results were obtained must be given in full. Two prizes of 30 dols. and 15 dols. each for the best and next best six photomicrographs on the same subject in animal or vegetable histology. The photomicrographs are to be of the following amplifications, viz. : 50, 150, and 500, two of each. They are to be made by transmitted light and printed on albumen paper from untouched negatives. Two prizes of 30 dols. and 15 dols. each for the best and second best six lantern slides, illustrating some one biological subject. The slides must be accompanied by a full description of the methods of preparation of the specimens. The photographs and slides are to become the property of the Society. The papers, etc., must be submitted to the Committee before July 1st, 1893. The competition is open to members of the Society and to those who make application for membership before submitting their papers. The entrance and annual fees are 3 and 2 dols. respectively. All further particulars can be obtained of the Secretary, Prof. W. H. Seaman, 1424, Eleventh Street, Washington.

At the opening meeting of the R.M.S., Mr. G. C. Karop described a microscope made of aluminium by Messrs. Swift. With the exception of a microscope which had an aluminium stage, shown in the United States some months ago, we believe it is the first instrument practically made entirely of that metal. Of course, its extreme lightness is the chief characteristic, the weight being 2 lb. 13 oz., against 7 lb. 10 oz. of an exactly similar instrument made in brass. The adjustments and screws were the only parts in which aluminium was not used on account of certain difficulties inherent to that metal.

THE ROBERTSON CYANIDE BOTTLE (*American Naturalist*, xxvi., 1892, p. 352).—Prof. C. Robertson uses a wide-mouthed bottle with cork stopper. Out of the lower side of the cork he cuts a hole, into which is inserted a pill-box filled with cyanide. A dozen pin-holes are made in the bottom of the pill-box through which the fumes can pass into the bottle. The bottle can be easily washed out, and has many advantages, especially for flies, bees, and similar insects. It is to be preferred to the ordinary plaster-of-Paris cyanide bottle.

Correspondence.

PICRO-CARMINE STAIN.—In reply to Mr. B. Ives, I see that Squire, in his "Methods and Formulæ," recommends Ammonia Picro-Carmine, prepared as follows:—"Carmine, 1 grm.; Strong solution of Ammonia, 3 cc.; Distilled Water, 5 cc. Dissolve the carmine in the ammonia and water with a gentle heat; then add saturated aqueous solution of Picric Acid, 200 cc.; heat to boiling and filter. This solution gives good results when used as follows: Take a section which has been rinsed in distilled water and lay it out flat on a glass slide, drain off the superfluous water, then pour on to the section several drops of the Picro-Carmine Solution, warm the slide over a spirit-lamp to a heat that can be borne by the hand when touched with the glass (if the section be too strongly heated, it will shrivel), keep it about this temperature for five or ten minutes, remove the excess of stain by tilting the glass and wiping it with a cloth or filter paper, leaving some of the stain in the section, then place one or two drops of Formic Farrant upon the section, and apply the cover-glass. The staining of the section is much improved after it has been mounted two or three days and exposed to daylight. A section of skin gives the most striking results by this method. Nuclei and the transverse muscular fibres stain red, the remainder yellow."

W. H. B.

Will any microscopist kindly tell me the cause of the clouded appearances which occur in apochromatic objectives? I think it is known as the *apochromatic disease*. If the objective is sent to the makers, they return it as good as new; but the question as to the defect and its cause is quietly shelved.

T. ASHCHURCH.

Reviews.

GUIDE TO THE SCIENCE OF PHOTO-MICROGRAPHY. By Edward C. Bousfield, L.R.C.P. London. 8vo, pp. xv.—174. (London: J. & A. Churchill. 1892.) Price 6s.

This is a second edition, entirely re-written and much enlarged. The author gives special consideration to the difficulties usually met with in histological and bacteriological work, and has introduced a new section, in which he deals *in extenso* with the method of photographing cultures; a number of good illustrations of photo-micrographic cameras, etc., are given. The book will unquestionably be found of much assistance to those interested in the subject.

MEDICAL MICROSCOPY: A Guide to the use of the Microscope in Medical Practice. By Frank J. Wethered, M.D. London. Crown 8vo, pp. xx.—412. (London: H. K. Lewis. 1892.) Price 9s.

Some very useful hints for the microscopist are given here, whether or not he belongs to the medical profession, but for the medical practitioner or student it will prove invaluable. It treats of the most simple methods of preparing micro-sections, and of the examination of urinary deposits, sputa, blood, etc. Elaborate methods are also given for the examination of foods and bacteriological studies. There are upwards of 100 illustrations.

UNTERSUCHUNGEN UBER MIKROSKOPISCHE SCHAUME und das Protoplasma, von O. Bütschli. 4to, pp. iv.—244. (Leipzig: Wilhelm Engelmann. 1892.) Price 24 marks.

This is an exposition of the author's "foam theory" of the structure of protoplasm. He first gives a very elaborate investigation into the formation and properties of microscopical foam, which may be produced in various ways, but after many experiments the best results were obtained from a mixture of olive oil and carbonate of potash. The fine froth thus produced simulates the movements of protoplasm in a remarkable manner; when a drop is examined upon a warm stage under the microscope, characteristic amœboid changes take place, and vigorous currents are set up. The explanation of the cause of these movements are given. Then follows a description of the structure of protoplasm in various organisms, and a critical examination of the various hypotheses which have been advanced to explain vital action. The author is of opinion that the structure of protoplasm corresponds to that of ordinary foam, with this difference, that the minute cavities in protoplasm are filled with a watery fluid instead of air, as in the foam. An exhaustive bibliographical list of works consulted is appended. The book is illustrated with six litho-plates and 23 wood engravings.

DIE NATURLICHEN PFLANZENFAMILIEN. By A. Engler and K. Prantl. Parts 72, 3, 4, 5. (London: Williams and Norgate. Leipzig: Wilhelm Engelmann.)

In these four parts thirteen orders are described, and very capably illustrated by 98 woodcuts, containing 709 figures.

BRITISH FUNGUS-FLORA: A Classified Text-book of Mycology. By George Massee. In three vols. Vol. I. Crown 8vo, pp. xii.—432.

This volume deals exclusively, and somewhat exhaustively, with the Basidiomycetes groups of fungi. The opening chapter gives an account of the nature and origin of Fungi. There are several woodcuts, illustrating more than 100 species.

GLIMPSES INTO NATURE'S SECRETS : Or Strolls on Beach and Lawn.

AMIDST NATURE'S REALMS : A Series of Zoological, Botanical, and Geological Essays. By Edward Alfred Martin. Crown 8vo, pp. xii.—131, and xii.—157. (London : Simpkin, Marshall, & Co., and Raithby, Lawrence, and Co. 1892.) Price 2s. 6d. each.

Two exceedingly instructive and well illustrated books ; each is divided into two parts, the subjects of the first being :—I., By Shore and Shallow ; II., Rock Written Stories. Those of the second are :—I., Life in the Living Present ; and II., Annals of a Far-away Past. The papers are interestingly written, and we heartily echo the author's hopes that they "may act as a key to unravel some of the secret wonders of nature."

VEGETABLE WASPS AND PLANT WORMS. By M. C. Cooke, M.A., LL.D., A.L.S., etc. Crown 8vo, pp. viii.—364. (London : Society for Promoting Christian Knowledge. 1892.) Price 5s.

Dr. Cooke here gives us a popular history of Entomogenous Fungi, or Fungi Parasitic on Insects. The first chapter of this interesting book treats of Entomogenous Fungi, and the four groups under which these fungi are arranged. In succeeding chapters they are arranged according to their host, under their various classes, viz., Hymenoptera, Coleoptera, etc. There are a number of illustrations, showing the insects and their parasitic fungus. At the end of the book is a classified list of the Entomophytes and 4 litho. plates showing the different fungi magnified.

BEETLES, BUTTERFLIES, MOTHS, AND OTHER INSECTS. By A. W. Kappel, F.L.S., F.E.S., etc., and W. Emont Kirby. Foolscap 4to, pp. 182. (London : Cassell and Co. 1892.) Price 3s. 6d.

A simple and popular guide to the collection and arrangement of insects. It explains the classification of insects, and describes their Metamorphoses, Habits, and Haunts. Directions are given for collecting insects and preparing them for the cabinet. There are 12 coloured plates and many wood engravings.

ANIMAL RIGHTS considered in relation to Social Progress. By Henry S. Salt. Crown 8vo, pp. x.—162. (London : G. Bell and Sons. 1892.) Price 2s.

The author declares his object in writing this book to be to set the principle of animals' rights on a consistent and intelligible footing. He is, of course, a strong opponent to vivisection, and as such we fear his zeal sometimes oversteps his consistency.

THE STUDENT'S HANDBOOK OF PHYSICAL GEOLOGY. By A. J. Jukes-Browne, B.A., F.G.S., etc. Second edition, revised. Crown 8vo, pp. xiii.—666. (London : G. Bell and Sons. 1892.) Price 7s. 6d.

This is exactly what we consider a "handbook" should be. It is written in an easy and interesting manner. Part 1 treats of Dynamical Geology :—I.—Changes produced by the Influence of Internal or Subterranean Causes ; II.—Those produced by agencies which operate on the surface of the Earth's Crust. Part 2.—Structural Geology. Part 3.—Physiographical Geology. There are two plates and upwards of 200 woodcut illustrations.

A TEXT-BOOK OF ELEMENTARY BIOLOGY. By H. J. Campbell, M.D.Lond. Crown 8vo, pp. iii.—284. (London: Swan Sonnenschein and Co. 1893.) Price 7s. 6d.

This volume gives a concise account of some of the more important facts of Biology. It treats at some length the subjects of Protoplasm, Cells, Cell-Division, Reproduction, and the Early Stages of Development. This book, which will prove of great assistance to the student, contains 136 very excellent illustrations.

LIGHT: A Course of Experimental Optics, chiefly with the Lantern. By Lewis Wright. Crown 8vo, pp. xvi.—391. (London: Macmillan and Co. 1892.) Price 7s. 6d.

This is a second edition of this valuable work. The author treats his subject in a thoroughly masterly manner, and places before the mind of his readers, through a course of experiments, the *physical realities* which underlie the phenomena of Light and Colour.

There are 10 full-page plates, several of them beautifully printed in colours, and upwards of 200 wood engravings.

TEXT-BOOK OF PETROLOGY. By Frederick H. Hatch, Ph.D., F.G.S., etc. Crown 8vo, pp. viii.—222. (London: Swan Sonnenschein and Co. 1892.) Price 7s. 6d.

In this work we have briefly described the mineral constituents and internal structures of the Igneous Rocks—their mode of occurrence at the surface, and their origin beneath the crust of the earth. The various chapters treat of—Mode of Occurrence, Structure, Composition, The Constituent Minerals of the Igneous Rocks, and their Classification and Description. There are 86 capital engravings, mostly illustrating the microscopic appearance of Rock Sections.

TIME AND TIDE: A Romance of the Moon. By Sir Robert S. Ball, LL.D., F.R.S., etc. Fscap. 8vo, pp. 192. (London: Society for Promoting Christian Knowledge. 1892.) Price 2s. 6d.

This very interesting little book, now in its second edition, contains two lectures delivered in the theatre of the London Institution in Nov., 1888. The theory of the tides is explained in a practical and understandable manner, and the illustrations are good.

THE GRAMMAR OF WOOD-WORK. By Walter E. Degerdon. (London: Macmillan and Co. 1892.)

This most useful work consists of a graduated system of manual training for Elementary, Secondary, and Technical Schools, designed for the pupils of the Whitechapel Craft School. It is divided into 21 lessons. The working drawings are admirable, showing the finished work from all its aspects. The instructions are short, but very concise and to the purpose. Size of page, 11½ in. square.

HOW TO MAKE COMMON THINGS. By John A. Bower. Crown 8vo, pp. 240. (London: Society for Promoting Christian Knowledge. 1892.) Price 3s. 6d.

This is just the very book for boys. It tells how to make a Hat-Rail, a Set of Bookshelves, or a Picture-Frame, How to Bind Books, How to Make some Useful Electrical Appliances;—in fact, how to do nearly everything that a handy boy wants to know. There are 150 illustrations.

WOODWORK, Carpentry, and Joinery. By Thos. C. Simmonds. Crown 8vo. (London: Bemrose and Sons.) Price 1s.

This little book briefly describes the methods of using the various tools and of making joints, etc.

THE STEAM-ENGINE CATECHISM, with Supplement. By R. Grimshaw, M.E., etc. 16mo, pp. 194 and 220. (New York : John Wiley and Son. 1891.) \$2.00

A series of direct practical Answers to direct practical Questions, mainly intended for young engineers and for examination questions. That this work is in its tenth edition speaks very forcibly for its usefulness. There are good indices to each part and several diagrams.

ATLAS OF COMMERCIAL GEOGRAPHY. By H. de B. Gibbins, M.A. (Edinburgh and London : W. and A. K. Johnstone.) Price 5s.

This compact little Atlas (size of page, 5in. by 7½in.) contains 48 maps, with explanatory letterpress. The maps of the continents and the British Isles are coloured Geologically and Physically ; the others are coloured Physically. In the letterpress descriptions, special prominence is given to the European Products, Industries, Trade Highways, Centres of Population, and Manufactures.

ARNOLD'S Abridged P.T.'s Year-Book of Memory Maps, Bk. I. (London : Simpkin, Marshall, and Co. Leeds : E. J. Arnold.) Price 1s. 4d. Contains 18 maps, with instructions for map-drawing. A useful little book.

CHEMISTRY. Part II., Inorganic and Organic. Crown 8vo, pp. 64. (Edinburgh : E. and S. Livingstone.) Price 1s.

We recommend these little "Catechism Series" books to the student, for we feel sure they will help to refresh his memory, especially if about to pass an "exam."

ARITHMETICAL CHEMISTRY, Book B. By C. J. Woodward, B.Sc. Crown 8vo, pp. 132. (London : Simpkin, Marshall, and Co. Birmingham : Cornish Bros. 1892.)

This is a new and entirely re-written edition of this work, in which many important additions have been made.

NOTES ON THE CLINICAL EXAMINATION of the Blood and Excreta. By Sidney Coupland, M.D., F.R.C.P., etc. (London : H. K. Lewis. 1892.) Price 1s. 6d.

Instructions for clinical and microscopical examinations are plainly given in this little book, which is of course intended for the use of the physician.

ILLUSTRATED AMBULANCE LECTURES. By John M. H. Martin, M.D., etc. Third edition. Crown 8vo, pp. xvi.—142. (London : J. and A. Churchill. 1892.) Price 2s.

A series of six lectures, given under the auspices of the St. John Ambulance Association. Lecture 1.—The Human Body and its Construction ; 2.—Hæmorrhage or Bleeding ; 3.—Fractures ; 4.—Shock or Collapse ; 5.—Method of lifting and carrying the Sick and Injured ; 6.—Nursing.

These lectures are nicely illustrated.

EPIDEMIC INFLUENZA : A Comparative Study in Statistics. By F. A. Dixey, M.A., M.D. 8vo. (Oxford : The Clarendon Press. 1892.) Price 7s. 6d.

The work before us is the result of an extended investigation into the statistical materials which have accumulated under the direction of the Registrar-General. These statistics are here arranged and grouped together in a compact form. There are 22 Tables and 11 Diagrammatic Charts.

A PRIMER OF THE ART OF MASSAGE for Learners. By Dr. Stretch Dowse. Fcap. 16mo, pp. 151. (Bristol: John Wright and Co. London: Simpkin, Marshall, and Co. 1892.) Price 2s.

Very plain and explicit directions are given for those who wish to make themselves acquainted with the general principles of the various modes of applying energy to the human body by means of the hands.

PRINCIPLES AND PRACTICE OF BANDAGING. By Gwilym G. Davis, M.D. 8vo, pp. xi.—61. (Detroit, Mich., U.S.A.: George S. Davis. 1891.) Price \$3.00.

This book goes thoroughly into the subject of bandaging, and describes—I., The Roller Bandages; II., The Tailed Bandages or Slings; and III., The Handkerchief Bandages. There are 23 plates, containing in all 172 figures, showing the various methods of using the bandage. The information in the letterpress is very explicit.

OPHTHALMIC DISEASES and Therapeutics. By A. B. Norton, M.D. 8vo, pp. 555. (Philadelphia: Boericke and Tafel. 1892.) Price 17s. 6d. net.

This is a text-book on Ophthalmology, in which special attention is given to the homœopathic treatment of diseases of the eye. The author gives in a very concise manner all the essential features necessary to a thorough knowledge of the diseases of the eye, commencing with sufficient anatomy of the various structures to aid in an understanding of their diseases. The book contains 53 illustrations and 12 chromo-lithographic figures.

CONTRIBUTIONS OF PHYSICIANS to English and American Literature. By Robert C. Kenner, A.M., M.D. pp. 93.

ACNE AND ALOPECIA. By L. Duncan Bulkley, A.M., M.D. pp. 85. (Detroit, Mich., U.S.A.: Geo. S. Davis. 1892.)

Volumes of the Physician's Leisure Hour Series. These books are well written, handsomely got up, and where necessary well illustrated. Price, in paper covers, 25c.; in cloth gilt, 50c. They are published monthly.

PUBLIC HEALTH PROBLEMS. By John F. Sykes, B.Sc., M.B. Crown 8vo, pp. xii.—370. (London: Walter Scott. 1892.) Price 3s. 6d.

The author states very forcibly some of the essential points in evolution, environment, parasitism, prophylaxis, and sanitation, bearing upon the preservation of public health. Part I. treats of Internal and External Influences upon Health; II.—Communicable Diseases; III.—Defensive Measures against them; and IV.—The Urban Dwelling. There are several illustrations.

THE BOYS' OWN BOOK OF HEALTH AND STRENGTH. By Gordon-Stables, M.D., C.M., etc. Crown 8vo, pp. 238. (London: Jarrold and Sons.) Price 2s. 6d.

We all know Dr. Gordon-Stables' manner in writing for boys. The book before us is written in his best style, and is full of plain and valuable advice for old as well as young boys; there are several plates.

AROUND THE ROMAN CAMPAGNA. By George E. Thompson. Crown 8vo, pp. viii.—156. (Liverpool: Edward Howell. London: Simpkin, Marshall, and Co. 1893.) Price 4s.

In this most interesting book Mr. Thompson takes the reader by easy stages around the Roman Campagna; he describes his visits to the various places of interest in a quaint and very amusing manner; indeed, having begun to read the book, you cannot leave it until you have read it all. There are six capital photo-illustrations.

BOOK OF DELIGHTFUL AND STRANGE DESIGNS, being 100 fac-simile Illustrations of the Art of the Japanese Stencil Cutter. Oblong crown 4to. (London : Leadenhall Press.) Price 6s.

Upwards of 100 fine designs in stencil work, as used by the Japanese, are given here. All the white parts of the design are intended to be cut out. A specimen original cut-out stencil is given as a frontispiece. Many of the designs are very handsome and a clever person will find them very useful.

CHINESE STORIES. By Robert K. Douglas, 8vo, pp. xxxvii.—348. (Edinburgh & London : W. Blackwood and Son. 1893.) Price 12s. 6d.

A series of stories reprinted from various sources, illustrating the popular literature of China, holding up, as it were, a mirror to the life of the people, and thus bringing home to us the fact that the human feelings are much the same on the banks of the Zang-tsze-Kiang as on those of the Thames. There are 8 full-page plates, and a great number of amusing illustrations in the text. The book is handsomely got up.

MATCHES THAT STRIKE. Edited by Rev. Chas. Bullock, B.D. Crown 8vo, pp. xii.—298. (London : *Home Words* Office.) Price 5s.

A collection of anecdotes which are well worth reading ; the editor calls them "Matches that Strike" because good anecdotes are always "striking"—sure to be remembered whatever else is forgotten ; and, like matches, however slight, they are often significant of important service.

NOTABLE WOMEN AUTHORS of the Day. By Helen C. Black. 8vo, pp. xii.—312. (Glasgow : David Bryce and Son. 1893.) Price 10s. 6d.

A series of twenty-six biographical sketches, each being accompanied by a full-page fine Photo-mechanical portrait. These sketches give a pleasing insight into the home life of the ladies whose writings are so well known. Readers are thus brought face to face with the authors whose works they are daily reading. The volume is handsomely got up.

THE ANTIQUITY OF MAN, from the Point of View of Religion. By F. Hugh Capron. 8vo, pp. 98. (London : Elliot Stock. 1892.) Price 4/6.

It will be enough to say of this book that it is written in answer to Mr. S. Laing's "Modern Science and Modern Thought." Its three chapters treat of : I., The Scientific View of the Problem ; II., The Bible View ; and III., The two Views Reconciled.

DID MOSES WRITE THE PENTATEUCH AFTER ALL? By F. E. Spencer, M.A. Crown 8vo, pp. xii.—291. (London : Elliot Stock. 1892.) Price 6s.

The author firmly believes in Moses, but we think he takes a somewhat too scholarly view of the subject to be readily followed and appreciated by the general reader.

MUSIC AND MOTION. Action Songs for Little Singers. Edited by Alan Reid, F.E.I.S. 4to, pp. 66. (Paisley : J. & R. Parlanc.) Price 2s. 6d.

A collection of 80 original and favourite Songs, Musical Games, Marches, Rounds, etc., for young people, with pianoforte accompaniments.

THE NOBLE AND JOYOUS HISTORY OF KING ARTHUR.

THE BOOK OF MARVELLOUS ADVENTURES and other Books of the Morte D'Arthur. Edited by Ernest Rhys. Crown 8vo, pp xxiv.—325, xiii.—384. (London: Walter Scott.) Price 1s. 6d. each.

These two volumes of the "Scott Library" contain the complete text of the Morte d'Arthur, and, save for modernising of the old spelling and a few unimportant omissions, are a fairly faithful version of the 1634 edition.

CASSELL'S NEW TECHNICAL EDUCATOR is now published in Monthly parts, at Sixpence. Part I. contains papers on a great variety of useful subjects. These papers are well illustrated, and are written by gentlemen who thoroughly understand what they are writing about.

THE FIRESIDE PICTORIAL ANNUAL. 1892. Edited by Rev. Charles Bullock, B.D. Crown 4to, pp. 858. (London: *Home Words* Office.) Price 7s. 6d.

This very excellent Magazine is full of most entertaining reading; besides several continued tales, there are Biographical Sketches of Great Authors, chapters on Books of the Season, and a host of other interesting things, with a number of full-page and other illustrations.

THE ROSE-BUD ANNUAL. 1893. Crown 4to, pp. 192. (London: James Clarke and Co.) Price 4s.

A capital book for the little ones. It contains nearly 300 illustrations, with plenty of music and poetry.

THE DAY OF DAYS. Crown 4to, pp. 186. Price 2s.

HOME WORDS. Crown 4to, pp. 284. Price 2s. (London: *Home Words* Publishing Office. 1892.)

Two yearly volumes of these prettily illustrated and interesting monthly magazines so suitable for young people.

SINAI from the Fourth Egyptian Dynasty to the Present Day. By Henry Spencer Palmer. Fcap. 8vo, pp. 224. (London: Society for Promoting Christian Knowledge. 1892.) Price 2s.

An interesting account is given of the physical character and present inhabitants of the peninsula of Sinai as well as its past history.

BAND OF MERCY. Crown 4to, pp. 96. (London: S. W. Partridge and Co. 1892.)

This attractively got-up volume cannot fail to please all young people, being full of pictures and stories about animals. It is issued by the Royal Society for the Prevention of Cruelty to Animals.

THE ZOO. By the Rev. J. G. Wood and Rev. Theodore Wood, F.E.S. Fcap. 4to, pp. 100. (London: Society for Promoting Christian Knowledge. 1892.) Price 2s. 6d.

This is the third series of "The Zoo," full of pictures, both plain and coloured, of birds and animals, and is just the book to please our young friends.

THE ANIMAL WORLD. Vol. for 1892. (London: S. W. Partridge and Co.)

This interesting magazine is truly, as stated in its title, "An Advocate of Humanity." It is issued by the Royal Society for the Prevention of Cruelty to Animals, and is full of illustrations, which are for the most part very good.

FALLOWFIELD'S PHOTOGRAPHIC ANNUAL.—This Catalogue of Photographic Materials, Chemicals, and Apparatus, consisting of nearly 600 pages, is now in its thirty-sixth year.

STUDIES IN PHOTOGRAPHY. By John Andrews, B.A. Crown 8vo, pp. xiii.—202. (London: Hazell, Watson, and Viney. 1892.) 3s.

In this handsome little volume the author claims that photography should rank as an original art, and gives some practical remarks to assist the photographer who aspires to produce more artistic work. There are six good photo-mechanical plates.

PRACTICAL GUIDE TO PHOTOGRAPHIC and Photo-Mechanical Printing. By W. K. Burton. Crown 8vo, pp. xviii.—415. (London: Marion and Co. 1892.) Price 4s.

The second edition of this useful work has been very carefully revised and in many cases thoroughly re-written. The first half of the book is devoted to what is ordinarily called Photographic Printing. The latter part describes the various processes known as Photo-Mechanical Printing.

OTHER BOOKS RECEIVED.

HASTINGS AND ST. LEONARDS as Winter Resorts. By F. Augustus Cox, M.B.Lond. Crown 8vo, pp. 12. (London: John Heywood.)

THE BOOK OF REVELATION, Showing the Fourth Beast of Daniel, its Carcase, its Millennial and Jewish Fables—a sign of the end. By F. W. Christie, B.A., Camb., etc. Second edition, enlarged. Crown 8vo, pp. xvi.—594. (Liverpool: E. Howell. London: Simpkin, Marshall, & Co. 1892.) Price 7s.

SCRIPTURE PHOTOGRAPHS: Men in the Sunlight of the World. By James Elder Cumming, D.D. (Stirling: Drummond's Tract Dépôt.) Price 2s. 6d.

THE BERRIDGES OF SILVER LEA. By Sidney Watson. (Stirling: Drummond's Tract Dépôt.) Price 2s.

THE VISIBLE TO-BE: A Story of Hand Reading. Crown 8vo, pp. 133. (Leadenhall Press.) Price 3s. 6d.

THE AWFUL AND ETHICAL ALLEGORY OF DEUTERONOMY SMITH. Foolscap 8vo, pp. 68. (Edinburgh: E. & S. Livingstone.)

Sea-Water Aquaria.

BY R. LAWTON ROBERTS, M.D.

ILLUSTRATED BY MISS FLORENCE PHILLIPS.

Plates V. and VI.



THE illustration (Plate V.) represents the general plan of a small private aquarium, constructed on the principles (1) that *the water is circulated, but not changed*; and (2) that *the only vegetation present is such as develops from invisible germs existing in the water*.

In 1872, the late W. A. Lloyd wrote:—"The balance of existence between plants and animals in a streamless aquarium is never easy to maintain, and therefore amateurs have usually to choose between the meagreness of a tank with but very few and small animals in it, and one with so many that the destruction of the whole can be very quickly brought about by some small adverse circumstance; and there frequently is no choice between an aquarium with the water looking dull from an insufficiency of oxygen caused by too little light to act on the vegetation, and one with an exposure to so much light that the plants evolve so many spores (or seeds) that the water becomes opaquely turbid and of a greenish-brown hue. A stream of water in an aquarium, however, with the greater part of the water in a separate vessel, the latter containing no animals and never being exposed to light, and with a smaller part of the water containing the animals, and being fairly well illuminated, at once surmounts many difficulties, and is (so to speak) a fly-wheel which carries the whole machine of an aquarium over its dead points. But such an arrangement is expensive, and needs much attention in working it."

It is precisely "such an arrangement" that I have attempted to carry out in a practical manner, and the result of my efforts is figured in the accompanying illustration (Plate V.).

The tank (2) is strongly constructed, the base and ends being of slate, and the sides of plate glass; its *internal* measurements

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are as follows :—Height, 1 foot $3\frac{1}{2}$ inches (exclusive of slate rim) ; breadth, 1 foot $6\frac{1}{4}$ inches ; length, 2 feet $5\frac{1}{2}$ inches. It is placed on an exceedingly strong wooden *stand* (1), 2 feet 5 inches high.

Both the tank and stand were obtained from Messrs. Dick Radcliffe and Co., of 128 and 129 High Holborn, London.

The *cover of the tank* (4) at first consisted of a central piece of glass, supported by a perforated and painted metallic framework. This, however, was soon found to be extremely unsatisfactory, as the paint chipped off, the metal corroded, bits fell into the water, and the animals suffered in consequence. With a fountain playing and the metal frame being continually wet by the spray, it can be readily understood how the water became fouled. So I had an entirely new cover (4) made by the Indiarubber, Gutta Percha, and Telegraph Works Co., Limited, of 54 Castle St., Liverpool. This consists of five pieces of glass, fitted into a framework of ebonite. The central piece of glass is over 6 inches broad, rather more than 12 inches long, and $7\frac{1}{2}$ inches above the level of the top of the tank, the other four pieces of glass sloping up to it.

The main portion of the *rockwork* in the *tank* consists of a porous substance called Tufa ; but other parts are constructed of slag, pumice, etc., fixed in cement, and in addition there are stones, shells, gravel, and, at one end of the tank, sand to the depth of $3\frac{1}{2}$ inches. I should mention that the sand was obtained from the sea-shore.

In one of the slate ends of the tank there are two circular holes, half-an-inch in diameter, both in the middle line, one being 13 inches, the other $7\frac{1}{2}$ inches from the base. I made these holes with an auger or centre-bit, which bored through the slate without any difficulty and without cracking it. I then got two corks to fit the holes and pierced each with a "cork-borer," so that a glass tube of one-third of an inch in diameter could be pushed through. One of the corks, through which I had pushed a short piece of glass tubing, I fitted tightly into the lower hole in the slate ; and to the outer end of the glass I secured a piece of indiarubber tubing, which runs into a large dark reservoir. This forms the *overflow tube* (11), so that the water in the tank remains at the level of $7\frac{1}{2}$ inches.

Into the uppermost hole in the slate another cork is fixed,

pierced by a glass tube (10), which is bent down to the base of the tank and passes along, more or less hidden by rockwork, to near the centre, where it is twisted up for about nine inches, and ends in a very finely-drawn fountain-jet (5). Outside the tank the glass tube is connected by a piece of indiarubber tubing (9) with a fountain reservoir (6).

With regard to the glass fountain tube, this necessarily is bent three times in the vertical plane, and also twice horizontally on account of the irregularities of the rockwork in the tank ; and as considerable care was needed in the fashioning of the finely-drawn jet, I thought it best to obtain three or four tubes (in case of breakage) ready shaped, from skilled manipulators.

The fountain tube in use, therefore, and some duplicates of the same, were supplied to me by Messrs. Townson and Mercer, of 89 Bishopsgate Street Within, London, E.C.

The *fountain reservoir* (6) is supported on a *metal stand* (7), 5 feet 6 inches in height, consisting of a strong rod with a circular flat plate at the top, and three stout feet, which are screwed to the floor.

As for the *stand* (7) it really is an old music-stand, altered by a local smith for the purpose to which it is now applied.

The *fountain reservoir* (6) is a glass "aspirator." It measures about 1 foot 9 inches in height, inclusive of the narrowed and raised mouth, and nearly 1 foot in breadth, and holds 5 gallons of water. At the base of the aspirator is a small glass stopcock (8) communicating by indiarubber tubing (9) with the fountain tube (10) in the tank. Fixed into the mouth of the aspirator (6) is also the open end of the delivery hose (15) of a pump (13).

The *large dark reservoir* (17) is a circular earthenware "mixing pan," over $2\frac{1}{2}$ feet in height, 2 feet in diameter, and made to hold 40 gallons. The "mixing pan" came without any cover, but I had a flat one made of hard oak, with a sufficient opening to allow of the passage *into* the reservoir of the overflow tube (11), and the passage *out* of the reservoir of the rubber suction hose (16) of a pump (13). The suction hose (16) is 1 inch in diameter; the open end hangs in the water of the reservoir, and as the hose passes through the cover over the side of the reservoir down to the pump, it acts as a syphon, and the pump always contains water.

The water is pumped up from the large dark reservoir into the fountain reservoir by means of a small *rotary pump* (12, 13, 14). A plan of this pump, showing both front and side views, is given in Miss Phillips' other drawing (Plate VI.). The corresponding portions of the pump in both side and front views are marked by similar figures. There is a strong wooden board (1), $8\frac{3}{8}$ inches broad, $1\frac{7}{8}$ inch thick, and 4 feet long; and on this is fixed a driving wheel (3), with a suitable handle (4), and lower down on the board is securely attached the actual pump (5). An india-rubber driving cord (6, 6) passes around the driving-wheel and a small "head" connected with the pump; and so by seizing the handle (4) and turning the driving-wheel, the pump is very easily worked.

The india-rubber hose used with the pump is an inch in diameter. The delivery hose (10) is fitted on to the upper opening of the pump (9), and is tightly fastened thereon by copper wire. The suction hose (7) is secured in the same manner on to the lower opening (8) of the pump. The driving-wheel (3) has a diameter of 15 inches, and the handle (4) is 4 inches in length. These measurements, with those of the wooden board already given, will, I think, give a general idea of the size of the entire concern.

Passing back again now to the drawing of the aquarium (Pl. V.) the pump (12, 13, 14) is seen in its actual position. The strong wooden board (14) is fastened securely flat against the wall (18) in an oblique or slanting direction, so that the driving-wheel (12) is at a convenient height for working. The actual pump (13) is quite near the floor, since it is necessary to ensure the proper working of such rotary apparatus for water to be always in it; and this is ensured by the syphon arrangement of the suction hose (16). It should be mentioned that all the parts of the pump in contact with the water are covered by vulcanite, and this is of course essential always where sea-water is in question, as otherwise the water would become impregnated with poisonous metallic impurities.

I have had this apparatus working for several months, and it acts admirably. In order to fill the fountain reservoir from the large dark reservoir, it is only necessary to turn around the driving-wheel at moderate speed for about a minute and a-half.

The water rushes in a full, steady, and continuous stream from the delivery hose into the fountain reservoir, as long as the driving-wheel is being turned. Once the fountain reservoir is filled, the fountain in the tank plays for six hours, the excess of water passing through the overflow tube into the large dark reservoir. I find that with very little attention—though this must be regular—the fountain can be kept playing and the water of the aquarium circulating for from fifteen to eighteen hours in the twenty-four.

The pump was made especially for me by Messrs. Leete, Edwards, and Norman, Limited, of 366 and 368 Euston Road, London, N.W. This firm appear to have made a speciality of the manufacture of patent rotary pumps for the circulation and aëration of public marine aquaria, and have supplied powerful machinery adapted to the purpose for aquaria at Plymouth, the Crystal Palace, and many other places.

An exceedingly useful instrument, in connection with an aquarium, is a pair of *long wooden forceps* for the purpose of feeding the animals and removing any dead creatures or other matters likely to foul the water. Forceps of this kind are in use at the Crystal Palace, and are made by Messrs. Aston and Mander, machine rule makers, Old Compton Street, London, W. I have a pair by me as I write, measuring $20\frac{1}{4}$ inches in length, each blade being half-an-inch broad and about quarter-an-inch thick, and the distance between the free thinned ends of the blades equalling about an inch and a quarter.

When first I commenced operations, I was in some doubt—living inland as I do—where it would be easiest to obtain *sea-water*. I finally decided to profit by the arrangements offered by the Great Eastern Railway Sea-Water Office, 122 Bishopsgate St. Without, London, E.C. From this source good sea-water can be obtained in three-gallon kegs, at the rate of sixpence a keg; unless four or more kegs are ordered, when the reduced rate is charged of fourpence half-penny a keg, or one shilling and sixpence for every twelve gallons. A deposit of four shillings and sixpence a keg is invariably required, but this is returned on application, providing the empty kegs are returned to the office carriage paid and in good condition.

It is well to remember that if there are difficulties in procuring

sea-water in a natural state, it can be made artificially. Gosse produced, in 1854, a formula for the purpose which answers admirably, viz.—Common table salt, $3\frac{1}{2}$ ounces (Avoir.); Epsom salts, $\frac{1}{4}$ ounce (Avoir.); Chloride of Magnesium, 200 grains (Troy); Chloride of Potassium, 40 grains (Troy). These salts to be dissolved in rather less than four quarts of ordinary drinking water, so that the solution attains a specific gravity of 1027. If the *hydrometer* shows a figure higher than 1027, then more water must be added; if under 1027 more of the salts are needed.

Perhaps the simplest method is to procure some of Southall's Aquarium Sea Salt, in each package of which is a measure and the directions:—"For sea-water of full strength add a gallon of water to each measureful of salt." For aquarium purposes, adjust the specific gravity with a hydrometer or gravity bubble, so that its specific gravity shall be 1027 at 60° Fah. This salt "was used in preparing sea-water for the Royal Aquarium, Westminster, and for the magnificent aquarium at Aston, Birmingham, where 200,000 galls. were in constant use."

It is an advantage, however, to obtain natural sea-water, if this is possible, since it contains myriads of microscopic animal forms, which multiply and serve as food for the creatures in the aquarium, and also countless invisible vegetable germs or spores which, under the influence of light, will develop into a low form of vegetation. Yet water from the sea-shore (shore-water), though suitable for animal life in a state of nature, is very frequently found at first to be quite unfit for use within the narrow limits of an aquarium. For example, the 100,000 gallons of "beautifully clear sea-water in the Crystal Palace Aquarium" was, when first received from the shores of Brighton, "neither well-coloured nor of high density, nor in any way fit for the maintenance of animals."

One can easily understand this. Shore-water is often more or less impure from the introduction of sewage, refuse, and other impurities from the land; but such deleterious matters are continually counteracted and destroyed by the air absorbed by the immense and ever-changing water surfaces exposed to the atmosphere through the agency of waves, tides, currents, and the natural movement of the sea; and this purifying process is enormously increased by the action of numberless healthy, growing, and vigorous seaweeds.

Put the same water within the confined limits of an aquarium, and the case is quite altered. There is no growing vegetation present, neither is there the unceasing movement of Nature, —nothing of any importance, in fact, to counteract and destroy the impurity of the water. Allow the water to remain in the aquarium without changing it, or, in other words, without introducing more impurities by the addition of fresh water, and affairs will right themselves naturally. Air is absorbed by the water surface in contact with the atmosphere, and vegetation of a low form grows from invisible germs existing in the water, with the result that the impurities at first present are gradually destroyed, and the water slowly becomes clear, pure, and fit to sustain animal life.

The late W. A. Lloyd, as far back as 1874, wrote very emphatically on these matters. He said, in an article in the *Popular Recreator* :—“ I have incidentally mentioned failures from the use of new *sea-water*, and I have known many persons to have lost (from this cause, without knowing it) animals at the seaside which they would not have lost inland. It is so always when there is an occasional renewal of water from the sea wherever the water is turbid, and the ill result is increased in proportion to the frequency of renewal. It is to this source that may be traced the too-small commensurate biological value of all public seaside aquaria built up till now, when their very large money-cost for erection and maintenance is remembered. That is to say, too much reliance has been by the constructors placed on the facilities which the position of such aquaria give for obtaining new sea-water, and that sea-water is almost always impure, and of much varying density at the shore. Animals may or may not live in such shore-water in the sea ; but it is a very different thing to living in the same water in the confined limits and measurelessly smaller aeration of an aquarium, whence, unlike as in the sea, they cannot escape if they find the water and other circumstances unfit for them.

The advantages of having a marine aquarium at the seaside consist in the ease with which some animals can be obtained without their being carried during a long and exhaustive journey, and in the saving of some of the cost of the first supply of sea-water. But, once obtained, that *first* supply should be the *last*, and

it should be stored in great dark reservoirs, and should be *circulated, but not changed*. . . . It is true that if a marine aquarium were to be set up where the sea-water is always clear and equally dense, as, for example, in some of the islands of the South Pacific Ocean, or even in some of our English Channel Islands, then the water could be drawn directly from the sea into the aquarium, and perhaps the animals in the latter would derive benefit from the microscopic food contained in the water not otherwise possible."

The Crystal Palace Aquarium was established on those principles which Lloyd insisted on as being correct ; in fact, it was constructed under the supervision and direction of Lloyd himself. The sea-water taken from the shore at Brighton was impure and unfit for animal life when it first arrived at Sydenham, and it was fouled still further—rendered poisonous indeed as regards the purpose for which it was intended—by the absorption of lime from the fresh cement used in the construction of the reservoir and tanks. Yet, in due course, the action of the air absorbed by the water, and of the vegetation which gradually developed in the tanks from invisible germs present in the water, brought about the purification of the latter, so that, to use Lloyd's expression, "the water has become what it now is by keeping it and using it." The water in the Crystal Palace Aquarium is the same that was brought in an impure state from Brighton about twenty years ago ; and it is kept circulating in the following manner :—There is a huge reservoir, placed underground, containing 80,000 gallons of water ; and the series of large aquarium tanks containing the living sea creatures contain 20,000 gallons more. The water is driven by powerful pumping apparatus, continuously, by day and night, from the reservoir through the whole series of tanks, and back again into the reservoir. In the course of circulation the water not only drops several inches from each tank into the next one, but is also forcibly driven from pipes in jets down to the bottom, or nearly to the bottom of each tank, by which means the thorough aëration of the water is ensured.

I might quote much additional evidence in support of the plan of *not* changing the water in an aquarium, but the limits of this paper will not allow it. When I first attempted to keep living sea creatures, I changed the water periodically, at intervals

of about a fortnight ; and I noticed (and in those days of ignorance wondered too) that the animals looked bad and sickly for a time after each change—and this though the sea-water supplied by the G.E.R. sea-water office is guaranteed to be taken a considerable distance from land, and therefore is proportionately pure.

Lloyd's suggestion that "where the sea-water is always clear and equably dense, as for example . . . in some of our English Channel Islands, then the water could be drawn directly from the sea into the aquarium," is about to be practically tested. A public sea-water aquarium, the result of private enterprise, is even now being established (in connection with fresh-water aquarium globes, a museum, library, and zoological laboratory) at Havre-des-Pas, Jersey. The sea-water tanks are constructed to hold from 4,500 to 5,000 gallons, and the water is to be pumped directly from the sea into the tanks, passing through them, and then running off waste. At least continual change of water will be kept up steadily for about sixteen hours out of the twenty-four, and it is thought that the animals, under the circumstances, will not suffer from the water being stationary during the remaining eight hours. This enterprise, in the light of Lloyd's experiences and his well-known views, is of the greatest possible interest, and is being anxiously watched.

When first I started my aquarium, I was of course quite alive to the need of having vegetation growing in the tank ; and, being acquainted with the interesting and beautifully illustrated works of Gosse, I was strongly impressed by the necessity of procuring young or full-grown sea-weeds for my purpose.

"*Ulva latissima*," says Gosse in his "Aquarium," "is probably the best of all sea-weeds for our purpose, and is one of the most easily procured on every shore." So the Sea Lettuce (or *Ulva latissima*) I got, and placed two or three pieces in my tank. The result was certainly striking, but not quite of the character that was anticipated and hoped for. The water became turbid, and covered with a soapy-looking scum ; two or three of the animals died, and it was only with much difficulty that any of the collection in the tank were saved.

Just about this time I met with a number of articles written by A. W. Lloyd, and in one of them I found the following

passage :—" And now, as I have poked fun at these antediluvian aquarium sea-weed gatherers, I should like to say something which is not fun. I have already mentioned that the collecting, transmission, and introduction of them was a very obvious application of the balancing arrangement of plants and animals, and there would have been nothing to say against it on the score of being lumbering, costly, or anything else, *if these sea-weeds would live in captivity* as well and as easily as the animals which these plants were supposed to keep in health. *But they would not live, they will not live now.* And then, to add to the provocation of the matter, they will sometimes live and thrive, perhaps one time in a hundred, or one time in a thousand, when one takes no pains at all with them, and die outright immediately when they are made the subject of goodness knows how much solicitude."

The fact is, as Lloyd found out for himself, that if the water (whether salt or fresh) is left in an aquarium without being changed, and exposed to light, a low form of vegetation (*algæ*) will develop on the rock-work and glass from invisible germs existing in the water ; and this vegetation—which grows naturally, and is adapted to the circumstances of an aquarium—is amply sufficient for all purposes, so that there is not the slightest necessity for the introduction of young or full-grown aquatic weeds from without.

So, after my failure with *Ulva latissima*, I made no further attempt to introduce and cultivate sea-weeds in my tank, but let the water rest for a time, exposed to a moderate light, not even circulating it, and of course not changing it. In a few weeks, sure enough, there developed on the glass, and on the rock-work, both green and brown vegetation. The green grows chiefly on the glass and the exposed portions of the rock-work which face the light from the window, whereas the brown growth appears on the submerged rock-work, and on parts more in the shade. This vegetation exists mostly in the form of a coating over the parts which it favours, but on the lower portions of the rock-work, tawny filaments, half an inch or so in length, shoot up in tufts, and from these, when the sun shines on them, streams of bubbles may be seen rising upwards through the water.

I cannot refrain from giving a quotation bearing on this sub-

ject from Lloyd's *Handbook to the Marine Aquarium of the Crystal Palace* (1872). It runs thus :—

“ Mrs. Thynne need not have sent from London to the sea for sea-weeds to revivify the water for her flagging corals, even though she for the first time thus intelligently applied the purpose of the vegetation in a marine aquarium ; as had she but exposed the water long enough to light, sea-weeds would inevitably have come, even in London, without their having been visibly put in—and they doubtlessly did come without being recognised, in her case. It was the same with Mr. Gosse, and with every other writer who recommended the putting masses of grown-up or even young plants into tanks. Such vegetation is very elegant, often as much so, and as interesting, as the animals themselves, but with the exception of the knowledge taught by Warington and Gosse, namely, that a few of the red *Algæ* can be grown in captivity in darkness or in much shade, or by the interposition of coloured media, it is not known how to systematically cultivate the green kinds, as *Ulva*, *Porphyra*, and *Enteromorpha*, or indeed hardly any others, whether they be brown, or red, or green. By chance, indeed, sometimes one here and there (and even difficult kinds occasionally, as *Delesseria*, *Laminaria*, and others) may be grown more or less well ; but as the reason is unknown, a repetition of success can seldom be had ; and, in fact, so uncertain are these *Algæ*, and so easily are they killed from a slight disturbance of condition, that sometimes an alteration of position of even the extent of a few inches in a tank, without the attachment or any other part being disturbed or injured, will cause a growing plant to die. It may be that some *Algæ* need the alternate exposure and submersion of tides, or the successive periods of rest and growth afforded by the cold and warmth of actual nature ; or possibly some require tidal actions, or the influence of the rain they occasionally get when the tide is out. Be that as it may, it is certain that with our present knowledge, the putting into an aquarium of masses of already grown sea-weed, especially the green kinds, whether they be young or old, or attached to stones or not, or of any ready-grown fresh-water plant, is not only very seldom attended with an after successful result, but so much positive harm is done by the decomposition arising from their

decay, that it is better to avoid them altogether, and to depend only upon that which gradually and naturally appears upon the rocks of the aquarium by the action of light, and which answers every chemical purpose; and the amount of growth of such vegetation can always be precisely regulated by the amount of light given to them—and so vigorously does it grow on places of its own choice, that its tendency is to increase too much when the light cannot be so far diminished without making an aquarium too dark for objects to be distinctly seen in it—and in such cases some animals, as the mollusk, the ormer (*Haliotis*), and the fish, the grey mullet (*Mugil*), are employed to eat it down to the very small quantity required to decompose the carbonic acid gas of even the largest aquarium.”

In my tank there are two Periwinkles (*Littorina littorea*), one Top (*Trochus umbilicatus*), three Chitons (*Chiton fascicularis*), and one Dog Whelk (*Purpura lapillus*); and all these are at work keeping down the vegetation. The Trochus seems happy enough and very busy; so it is interesting to note, on Lloyd's authority, that “the Top is of delicate organisation, and usually dies (in a streamless aquarium) instead of doing any work.” It is curious to see how clean the Chitons sweep the rock-work. The bare, raw-looking patches on the rock mark their track, and contrast strangely with the brown or green vegetation on either side. The Periwinkles work very spasmodically, sometimes adhering to the slate, close together, high out of the water for several days without moving; whereas at other times they are very active, clearing off the vegetation and following it down into the porosity of the rock-work, so as to leave little depressions or concavities. As to the Whelk, he progresses but very slowly; he keeps to a piece of rock-work in a corner of the tank, and either works very little, or does his business with great thoroughness, so long does he take to get over a small space. I notice that this creature sticks to the rock-work with great tenacity.

I have between thirty and forty other specimens, some of which are very fine and large, in my tank, and hope after a time to procure more. Two of these specimens I may briefly allude to, on account of the difficulty some have in keeping them alive in captivity. One is *Tealia Crassicornis*, the Dahlia Wartlet or

Crass. Of this gorgeous Anemone, Pennington says :—"It is one which may be kept with ease in an aquarium ;" but other observers have been led by their own experience to form quite an opposite opinion. Thus Gosse remarks :—"Beautiful as is the Dahlia, it is not a very frequent tenant of our aquariums, as it is one of the most difficult to keep. . . It appears to be little able to sustain extremes of temperature. The heat of summer is generally fatal to our captive specimens ; and a severe winter makes havoc among those which are in the enjoyment of freedom." And Bennett says that his "own experience goes to prove that they die in a few days."

I seem to be one of the lucky ones, for my large *Tealia* flourishes well, and I have had him for several months. In connection with this Anemone, it is interesting to note that Dique-mare observed :—"Of all the kinds of Sea-Anemones, I would prefer this for the table ; being boiled some time in sea-water, they acquire a firm and palatable consistence, and may then be eaten with any kind of sauce. They are of an inviting appearance, of a light shivering texture, and of a soft white and reddish hue. Their smell is not unlike that of a warm crab or lobster."

The other creature against which I was warned as one unlikely to live in captivity is *Anthea Cereus*, or the Opelet. But here again I have been successful, for the *Anthea* in my tank flourishes well. I had often noticed, when shore-hunting, that the Opelet seemed to thrive in shallow tide pools, exposed to the direct rays of the sun, and the water of which could only be replenished at high water. I also noted that Lloyd, in his *Handbook*, referring to the Anemones of the Crystal Palace Aquarium, remarked that "some of them are diurnal in their habits, however, notably *Anthea*, which likes much exposure to light, and does not fade, and has a tendency to close at night. . . The one in the collection least apt to close when touched is *Anthea*, and this is also the one most constantly open by day, and frequently apt to close at night ; the reverse of this rule being the one generally found to obtain among Sea-Anemones."

Accordingly, when my *Anthea* first arrived, I dropped it on a portion of rock-work only just covered by water, and fully exposed to the light, and the creature has flourished well. I have

also noticed the marked diurnal character of the animal, its condition of wide expansion by day, and its tendency to shrink at night. The colour of this Anemone is dependent, I believe, in considerable measure, on the amount of light to which it is exposed. The extremely vivid colouring—the bright green and red—present in some specimens, appears to be due to exposure to direct and powerful sunlight. If the same creatures are placed in positions subjected to less powerful light, then the colouring becomes correspondingly subdued.

It seems that this species has not been overlooked by epicures, since Johnson observed :—"Even the hot and peppery *Anthea* has its praise ; from it they prepare the dish called *Rastegna*, which is a favourite in Provence."

Gosse made several experiments in the way of preparing Sea Anemones for food. He did not think much of *Althea*, but achieved a great success with *Tealia Crassicornis*. He had these very carefully cleaned, every trace of slime and adherent particles being scraped off completely. They were then fried in egg and bread-crumbs, with the result that "all prejudice yielded to their inviting odour and appearance, and the whole table joined in the repast with indubitable gusto."

NOTE.—Since the above paper was written, I have received information regarding the susceptibility of *Tealia Crassicornis* to cold, which is quite contradictory to the experience of Gosse. A friend writes :—"As regards *cold* and its effect on Anemones, I had a large *Crassicornis* in a bowl, and it got frozen into a *solid block*, like a fly in amber. It thawed out next day and expanded beautifully, and was as hearty and well as ever."

EXPLANATION OF PLATES V., VI.

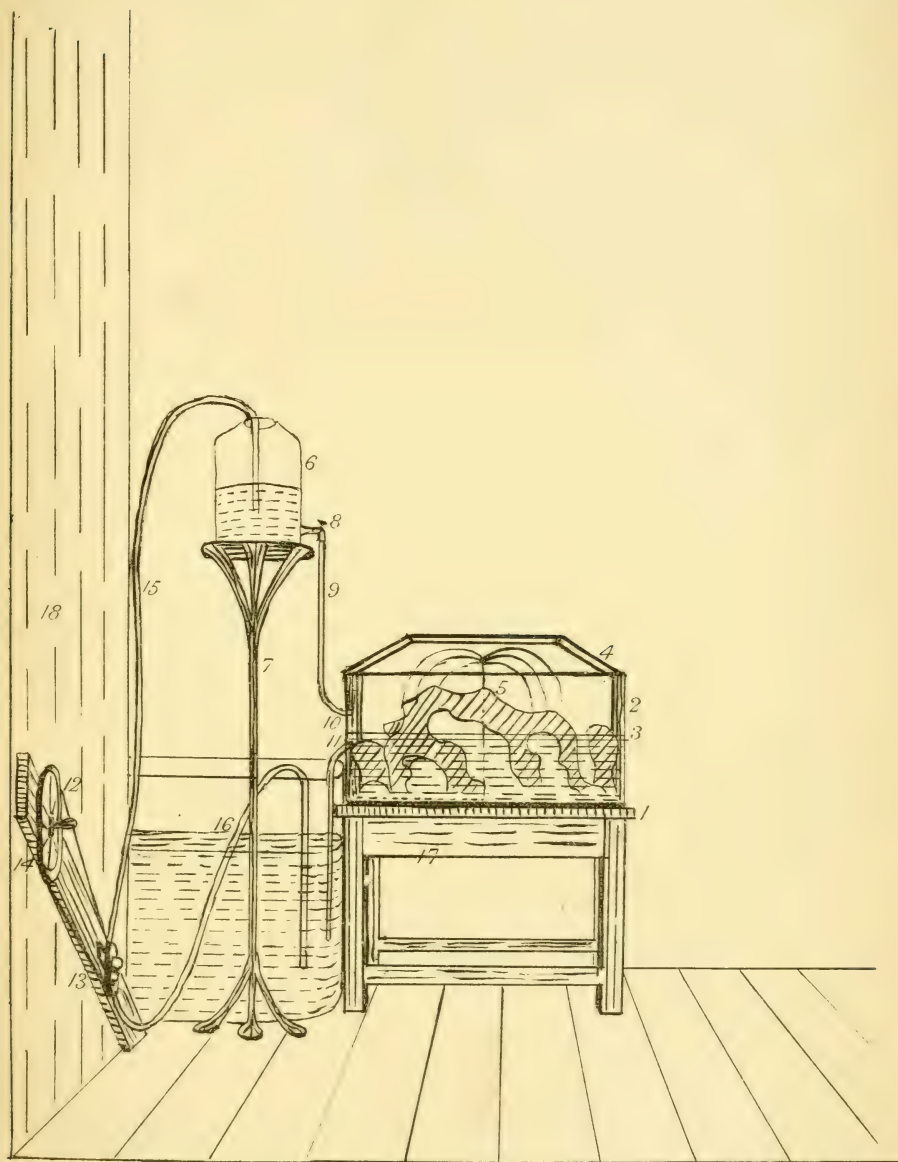
PLATE V.—Plan of a private Sea-water Aquarium.

Fig. 1.—Strong wooden stand.

„ 2.—Aquarium tank, sides of plate glass, base and both ends of slate.

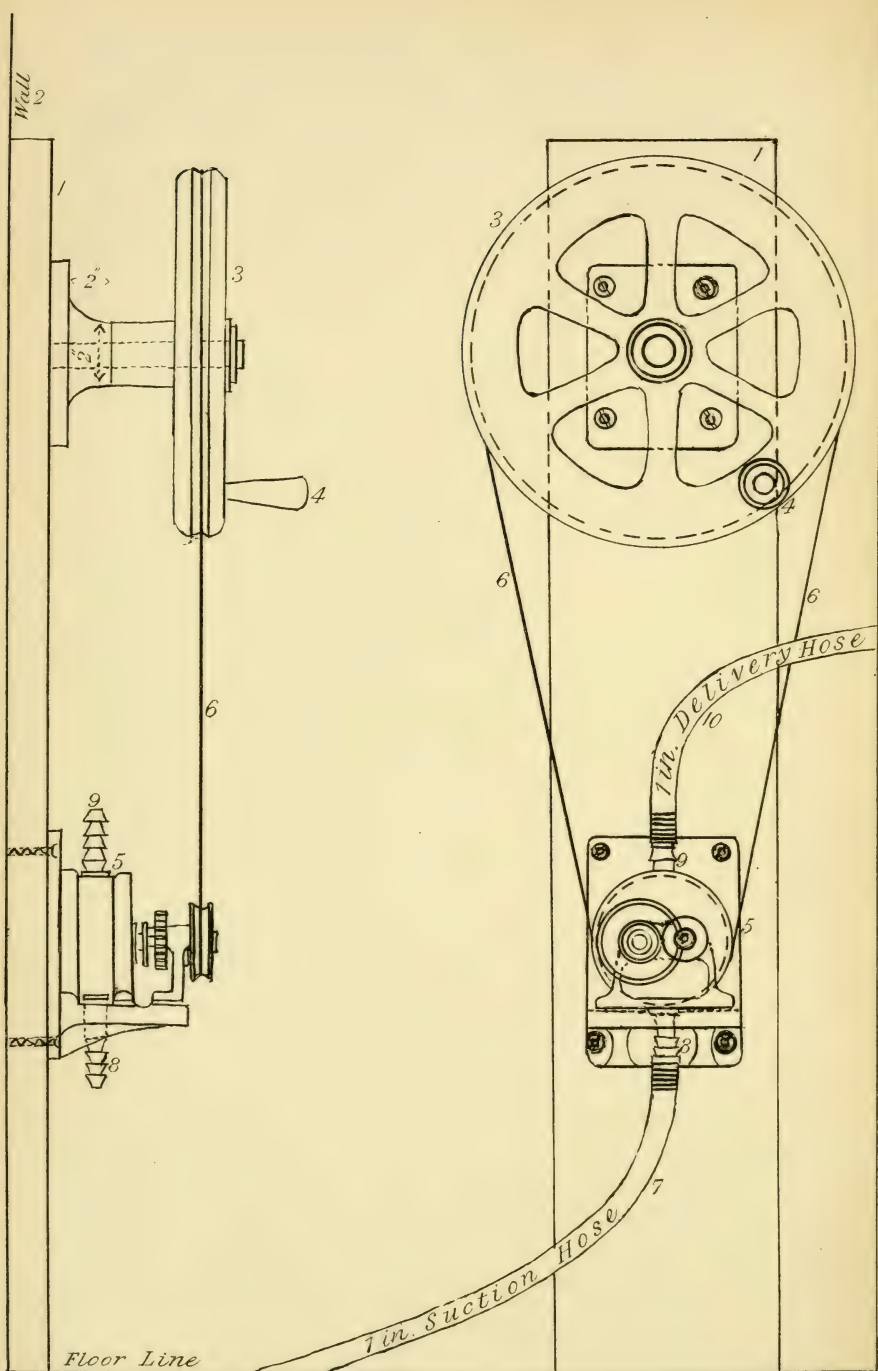
„ 3.—Surface of water in aquarium tank.

„ 4.—Aquarium tank cover of ebonite and glass.



F. Phillips Del. et Sc.

Private Sea-Water Aquarium.



F. Phillips Del. et Sc.

Rotary Pump, showing front and side views.

Fig. 5.—Fountain in aquarium tank.

- „ 6.—Glass vessel (or *Aspirator*) : acts as fountain reservoir.
- „ 7.—Iron and brass stand of fountain reservoir.
- „ 8.—Glass tap of fountain reservoir.
- „ 9.—India-rubber tube, leading from fountain reservoir to fountain jet.
- „ 10.—Glass fountain-jet ; dotted line shows the position in aquarium tank.
- „ 11.—Overflow glass pipe and india-rubber tube from aquarium tank leading into large earthenware reservoir.
- „ 12.—Driving-wheel of rotary pump.
- „ 13.—Rotary pump.
- „ 14.—Wooden board on which rotary pump and driving-wheel are fixed.
- „ 15.—1-inch delivery hose from pump into fountain reservoir.
- „ 16.—1-inch suction hose arranged as syphon from large earthenware reservoir to pump.
- „ 17.—Large earthenware reservoir (or Mixing Pan).
- „ 18.—Wall.

PLATE VI.—Plan of small Rotary Pump supplied by Messrs. Leete, Edwards, & Norman, Limited ; showing front and side views.

Fig. 1.—Strong wooden board.

- „ 2.—Wall.
 - „ 3.—Driving wheel.
 - „ 4.—Handle of driving wheel.
 - „ 5.—Rotary pump.
 - „ 6.—India-rubber driving cord.
 - „ 7.—1-inch suction hose.
 - „ 8.—Lower opening of pump.
 - „ 9.—Upper opening of pump.
 - „ 10.—1-inch delivery hose.
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PHOTOGRAPHY has thrown a curious light upon plant life. Photographs of a seedling have been taken every half-hour, with the result that sufficient change has taken place in the growth to be noted on the sensitive plate. A series of these photographs, placed in the zoetrope, give the impression of a stem growing under our very eyes. The statement is made that photography has demonstrated that even when asleep the plants were continually growing.

Polarised Light and its Applications to the Microscope.

PRESIDENTIAL ADDRESS BY G. H. BRYAN, M.A.

PART II.

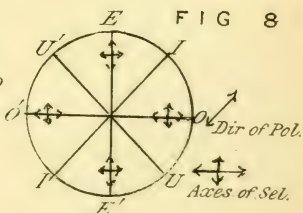
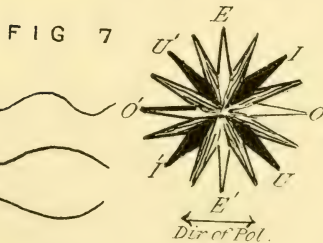
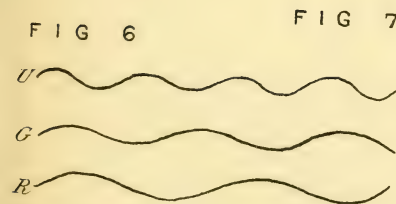
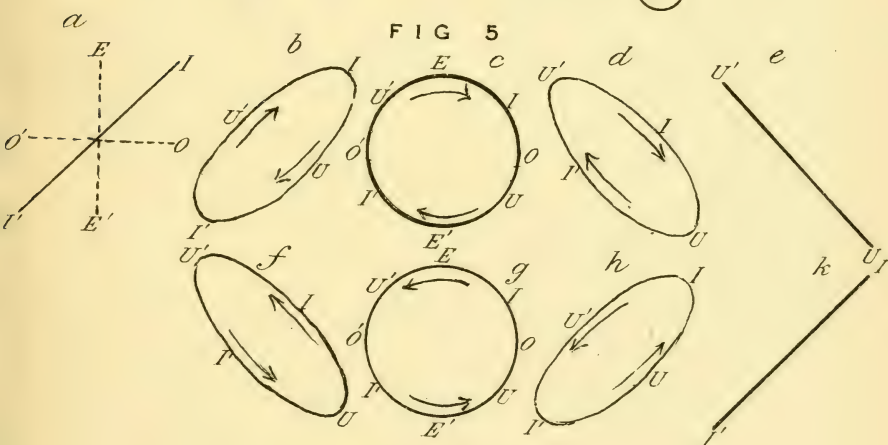
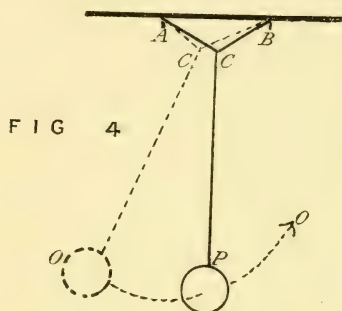
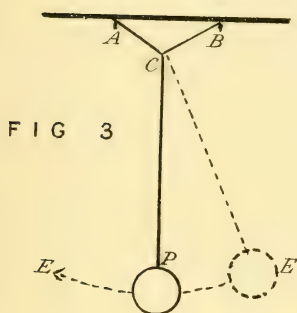
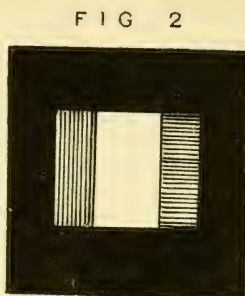
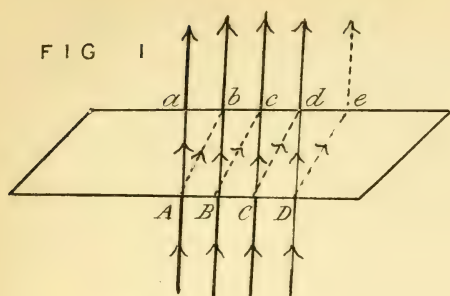
PLATE VII.

Doubly Refracting Polariscopic Objects.

MOST microscopical polariscopic objects are polariscopic on account of their possessing the property of double refraction, as described, for the case of Iceland spar, in the first part of this paper. Such is the case with many chemical crystals, thin films of selenite, certain woody tissues of plants, starch grains, sections of hoof and horn, many animal and vegetable hairs, and certain polyzoa. Crystals belonging to what is called the "cubic" system—of which common salt is an excellent example—are not doubly refracting and therefore not polariscopic. Glass, when properly annealed and unstrained, is not polariscopic, but it becomes polariscopic when it is strained by being subjected to great pressure or tension in one direction. Badly annealed glass sometimes becomes strained in the same way in the process of cooling, and it is then polariscopic. For this reason a polariscope is largely used in glass-works for testing whether the glass is properly annealed or not. Any articles which exhibit bright patches when the polariscope is arranged to give a dark ground are rejected.

Conditions necessary for Polariscopic Effects.—If a ray of ordinary unpolarised light fall on a section of a doubly refracting crystal, such as Iceland spar or selenite, it is split into two component rays, which are polarised in perpendicular directions, and these rays travel through the section in different directions and at different speeds. As in the case of Iceland spar, it will be convenient to call one of these rays the ordinary and the other the extraordinary ray,* and we shall call the directions in which these

* In many crystals, such as selenite, neither of the rays ought properly to be called an ordinary ray, but for our purpose the use of the terms "ordinary ray" and "extraordinary ray" will not be misleading.



two rays are polarised the *optic axes* of the section. We notice that *every section of a doubly refracting substance has two optic axes, and these are perpendicular to each other.*

If the section is not very thick, the beams of light will not have separated when they emerge, and they will re-combine to form a beam of unpolarised light. Thus, in Pl. VII., Fig. 1, the extraordinary ray from *A* and the ordinary ray from *B* unite to form an unpolarised ray, which emerges at *b*, and so on; only the extreme rays which emerge at *a* and *e* are polarised. If then, a crystal of Iceland spar is laid over a piece of black paper in which a sufficiently large square hole is cut, we shall see the appearance shown in Fig. 2. In the central portion the two images of the hole will overlap and the light will be unpolarised, while in the portions not common to the two images the rays will be polarised in different directions, as shown by the directions of the shading. When the images are examined with an analyser which is slowly rotated, first one image and then the other will be extinguished; but the central part will never be extinguished, so that the light which emerges from this part is unpolarised.

Hence, *if we examine a doubly refracting section with UNPOLARISED light, we shall not obtain any polariscopic appearances.* In other words, an analyser without a polariser would not be sufficient to form a polariscope.

Neither shall we obtain any polariscopic appearances if we examine the section with light polarised along EITHER OPTIC AXIS of the section. For if the incident light be polarised, say, along the optic axis of the extraordinary ray, it will be entirely refracted as an extraordinary ray, and will emerge polarised as it entered. If the polariser and analyser are "crossed," we shall still get darkness, and if the polariser and analyser are parallel we shall still get light just as if no object were interposed.

We are now left with only one alternative, namely—*To obtain polariscopic appearances, we must examine the section with light polarised in a direction more or less DIAGONAL to the optical axes of the section.*

Before proceeding further, the microscopist should verify this by the following experiment with the polariscope and selenite, even if he does not procure a rhomb of Iceland spar for the purpose of carrying out the experiments above described.

Adjust the polariser and analyser of the microscope so as to give a dark ground, and place a slide of selenite on the stage. On rotating the selenite into a certain position the beautiful colours will all disappear, leaving a black ground. If the selenite is rotated through a right angle from this position, the colours will again disappear (to perform the experiment properly, the microscope should be furnished with a rotating stage). In either position, one of the two optic axes of the selenite coincides with the direction in which the light is polarised by the polariser. On turning the polariser through a right angle, a white field of view will be obtained just as if there were no selenite. On the other hand, the brightest colours will be obtained when the selenite is rotated through 45° (half a right angle) from either of the aforementioned positions.

Thus, to obtain the best effects, the light should be polarised in a direction inclined at 45° to (i.e., half-way between) the directions of the optic axes of the section.

Pendulum Experiment.—To completely explain on the wave theory the changes which take place in a beam of polarised light as it travels through the section is very difficult. If we rely exclusively on theory, we cannot help finding ourselves face to face with mathematics, or something very like it. The following very pretty pendulum experiment, however, affords an excellent illustration of the phenomena, and as it requires no special apparatus I hope all my readers will perform it for themselves :—

Take two pieces of string (Pl. VII., Fig. 3), knot them together at C to form a Y , and attach the shorter ends A, B to a horizontal bar (such as that of a towel-horse) a few inches apart; or (what amounts to the same thing) tie a loop in the string and pass it over a narrow plank whose breadth is AB , so that it should hang in a Y as before. To the longer end (which should be a foot to 18 inches in length), attach any convenient weight P —say, $\frac{1}{4}$ lb.

If the weight be pulled out of the vertical to a point E in the plane of the strings (as in Fig. 3), it will swing to and fro along EE' after the manner of a pendulum attached to a fixed support at C , and the upper arms, AC, BC , will remain at rest. Next,

pull the weight aside in a direction PO perpendicular to the plane of the strings, so that this time all three strings swing about the line AB (Fig. 4). The weight will again begin to swing like a pendulum, but in a direction OO' *perpendicular* to its former direction, the chief difference being that it does not swing quite so rapidly as before—each oscillation takes a little longer. This difference in the rate of oscillation may be intensified—and so better exhibited—by making the upper strings (AC , BC) of considerable length and the lower string (CP) short. But for what follows it will be necessary to do the reverse, by arranging the strings so that the knot C is not more than an inch below the supporting-bar AB , while the lower string CP is of considerable length.

Now let the weight be pulled aside in a diagonal direction, say, at an angle of 45° , with the plane of the three strings. Let this direction be represented in Fig. 5 (*a*) by the diagonal line II' , where OO' , EE' , represent the directions in which the weight oscillated in the former cases. On letting the weight go this time it will begin by oscillating along the diagonal II' , but it will not continue to do so for any length of time. On the contrary, the motion will go through the series of changes represented in Figs. 5 (*a-k*). After a little while the weight will begin to swing a little from side to side of the diagonal II' , and will then revolve in an oval curve or ellipse as at (*b*). Gradually this ellipse will widen out until the weight revolves in a circle (*c*). After this the path of the weight will elongate along the diagonal UU' , and contract along II' (*d*), until a time comes when the weight swings to and fro along the opposite diagonal, UU' , as at (*e*). Subsequently, at (*f*) it will begin to revolve in an ellipse, but in the opposite direction to what it did previously. This ellipse will contract along the diameter UU' , and will open out along the diameter II' , and will again pass through the form of a circle (*g*). The path will then elongate as at (*h*), until at (*k*) the weight once more swings to and fro along II' , just as it did at starting. After this the same cycle of changes will be repeated. As long as the weight keeps swinging, the path in which it moves will keep changing periodically backwards and forwards from one diagonal to the opposite one, each time passing through all the intermediate forms of ellipses and circles shown in Fig. 5.

Application to transmission of Light through Crystals.—

Now, a precisely similar cycle of changes takes place, according to the wave theory, when a ray of light falls on a section of doubly-refracting material polarised in any direction other than along one of the two optic axes. Suppose that on entering the section the ray is polarised in the direction II' , inclined at 45° to the optic axes EE' , OO' . Then at the surface the ether is, of course, vibrating in straight lines in the direction II' . As the light penetrates into the section, the ether begins to vibrate in ellipses, and the light is said to be elliptically polarised. At a certain depth the light becomes circularly polarised. At double this depth (Fig. 5 *e*) it becomes polarised along the diameter UU' perpendicular to the original direction of polarisation. At treble the same depth it is again circularly polarised, but the ether is revolving in the opposite direction to what it was before. At four times that depth the light has gone through a complete cycle of changes, and is polarised in the same way as it was on entering. As the light penetrates further and further into the section, the same cycle of changes keeps recurring over and over again.

Now let the emergent light be examined with an analyser placed perpendicularly to the polariser, in the position known as "crossed." In this position it will transmit all vibratory motion of the ether in the direction UU' , but stop all vibration along II' . If the thickness of the section be such that the light emerges at the middle of the cycle of changes which we have just described—*i.e.*, in the stage represented at (*e*)—it will be polarised along UU' , and will be entirely transmitted by the analyser. The section will therefore appear bright while the field of view is dark. On the contrary, if the light happens to have undergone exactly one, two, three, or more cycles of changes, when it comes out it will be polarised as it was on entering, and the object as well as the field will appear dark. Unless, however, the light comes out exactly at the end of a cycle, it will be in a condition to be, at any rate partially, transmitted by the analyser, and the object will appear more or less brightly illuminated, and the perfectly dark background will render it conspicuous.

Suppose the analyser turned round parallel to the polariser, giving a bright background. It now transmits light polarised along

II' , and if the light happens to emerge from the section after undergoing an exact number of cycles of changes, the object as well as the field will appear bright. If, however, the light emerges in the middle of a cycle (Fig. 5 *e*), where it is polarised along UU' it will be quenched by the analyser, and the object will appear dark on the bright ground. For intermediate thicknesses the light is more or less reduced in intensity by the object, which therefore appears somewhat darkened, or at any rate less bright than the background. Now a slight diminution of brightness in a bright field is not so conspicuous as even a faint illumination where the surrounding field is perfectly dark, and for this reason, as every microscopist knows, objects which are sufficiently polariscopic to show up fairly well on a dark ground, frequently exhibit no very noticeable polariscopic appearances when the field is bright.

Comparison of the two phenomena.—It may perhaps be worth while to examine a little more closely the analogy between the oscillations in the pendulum experiment and the ether vibrations in the beam of polarised light, and to enquire *why* the same cycle of changes takes place in both. We have seen that the pendulum is capable of permanently oscillating about the knot C in the plane EE' of the strings, and that it is also capable of permanently vibrating at a slightly slower rate about the points A, B in a direction OO' perpendicular to that plane. When we pull the string aside in a diagonal direction we set both of these motions going at the same time, and the actual motion is a combination of the two. [For in Fig. 5 *a* we may suppose the weight pulled aside in the plane of the strings from P to E , and then in a perpendicular direction from E to I , and let go from I ; the first of these two displacements will start it oscillating in the direction EE' , and the second will start it oscillating in the perpendicular direction OO']. *But the quicker of the two oscillations gradually gains on the slower. This gain is the cause of the cycle of changes in the observed motion formed by the combination of the two oscillations.*

Whenever the pendulum has *simultaneously* performed an *exact* number of complete oscillations in the direction EE' and an exact number along OO' , it will return to rest at I , and will again begin to oscillate in exactly the same way that it did at the

beginning. This happens every time the quicker of the two motions has gained one whole oscillation on the slower, and then the complete cycle of changes of Fig. 5 has taken place in the series of curves traced out by the weight.

For example, suppose the pendulum vibrates in the plane of the strings once every second and in the perpendicular direction once in $1\frac{1}{2}$ seconds. Then, in 21 seconds, it performs 21 of the quicker oscillations or 20 of the slower, so that the former gain one oscillation over the latter. If, then, the weight be pulled aside in a diagonal direction, its motion will undergo the complete cycle of changes of Fig. 5, and again swing in the same diagonal after 21 seconds. In half this interval (or $10\frac{1}{2}$ seconds) it will vibrate in the opposite diagonal to that in which it started, as at (*e*).

Reverting to polarised light, we have seen that the doubly-refracting section is capable of transmitting rays of light polarised along either optic axes without altering their character. There are two such rays—*i.e.*, those which we have called the ordinary, and the extraordinary rays—and they travel through the substance at somewhat different rates. Hence, as they penetrate further and further into the substance, *one of them gains slightly on the other*, just as one oscillation gained on the other in the pendulum. Hence, remembering that the rays really consist in vibrations transmitted from point to point through the ether, we see that the circumstances of the case are exactly analogous to those of the pendulum experiment. It necessarily follows from this analogy that the changes in type of the light *at different depths*, when the ray enters the section polarised at an angle of 45° with the optic axes, exactly reproduce the changes of type in the pendulum oscillations *at different times*, when it is started swinging diagonally.

Another consequence of the analogy is that in the polariscopic section *the complete cycle of changes takes place when the light-vibrations in one of the rays have gained a whole oscillation over those in the other. When the gain is half an oscillation, half a complete cycle has taken place, and the polariscopic appearances are then most marked.*

The Grapho-Prism and the Technique of Drawing Microscopic and Macroscopic Objects.

BY FREDERICK GAERTNER, A.M., M.D., Pittsburgh, Pa., U.S.A.

WITH the assistance of the Camera lucida or Grapho-prism any microscopist possessing average skill in the use of the pencil, may, with comparative ease and perfect accuracy, reproduce the outline and principal markings of the

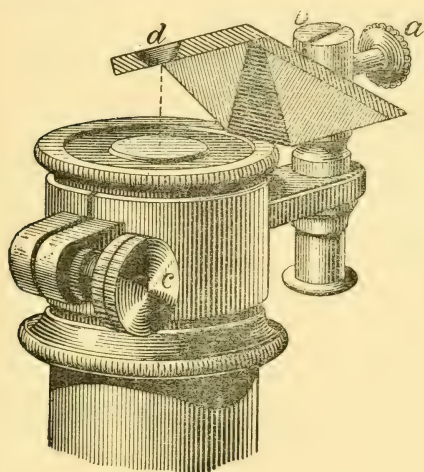


Fig. 18. —Zeiss' Grapho-Prism.

object under the microscope upon a sheet of paper lying beside his instrument. The simplest and most successful drawing-prism is that of Zeiss (Fig. 18). This is closely followed in merit and popularity by that of Nachet (Fig. 21), then that of Abbe (Fig. 22), Oberhauser (Fig. 23), and those of Nobert and many others, all working upon the same principles.

The following remarks and illustrations will explain and demonstrate the principle of this kind of drawing apparatus. If the glass plate—*gl.*, Fig. 19—stands at an angle of 45° with the axis of the eye, the rays from the object (*o*)—which on their part also form an angle of 45° with the glass plate, according to the position of the eye—will be reflected, and the picture of the object will be seen in a position which will also form a right angle with that of the object. If *m.* (Fig. 19) is the cylinder of the microscope and *p.p.* the piece of paper, in this case the eye will see upon the paper lying beside the microscope, at *o'*, the picture which is brought about by the trans-

parent condition of the glass plate, gl . In this case we say that the picture is projected; but if we place a prism, p . (Fig. 20), upon

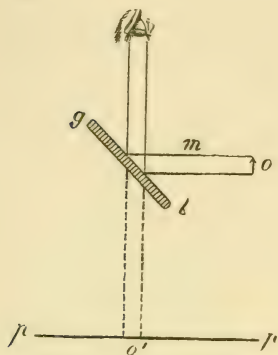


Fig. 19.

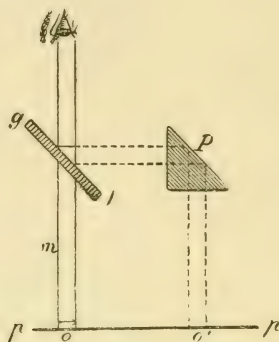


Fig. 20.

the same level with the glass plate, gl . (Fig. 3), and o . is the object under the microscope, m ., standing in a vertical position—the glass, gl ., forming an angle of 45° with the axis of the eye and standing upright over the ocular—we will then see the picture at o' projected upon the paper, pp . Upon this principle is also constructed the drawing-prism of Nachet (Fig. 21). In this apparatus a prism is employed in place of the glass plate, while a second prism moves upon its own axis so as to bring the reflecting surfaces at different angles.

The use of a drawing-prism is obvious, as soon as the drawing-prism has been placed upon the ocular and adjusted by means of a ring or other attachment. The drawing of a picture, whether of a *microscopic* or *macroscopic* nature, nowadays is considered a scientific achievement, especially so when the sketch is made by “free-hand.”

In the drawing of a macroscopic object (gross appearance) the principal considerations are the direct measurements, and these can easily be obtained by the use of circles, lines, rules, strings, and other metrical appliances on the one hand, and on the other by the use of a camera, photographic apparatus, magic lanterns, etc. etc. Of course (in this case) the object aimed at is, first, to

secure only the outlines of the macroscopic picture, and then, when this has been accomplished with only moderate artistic dexterity, ingenuity, and taste, one can readily reproduce a fac-simile or likeness ; but where pure freehand work is required—such as we observe in some of the ancient oil paintings, *e.g.*, those of Rubens, Rembrandt, Raphael, and others—the operator must rely entirely upon his own artistic skill and taste. By repeated measurements one will be able to obtain accurate and artistic results, such as we now see in some of the most approved, artistic oil paintings, water-colours, crayons, etc.

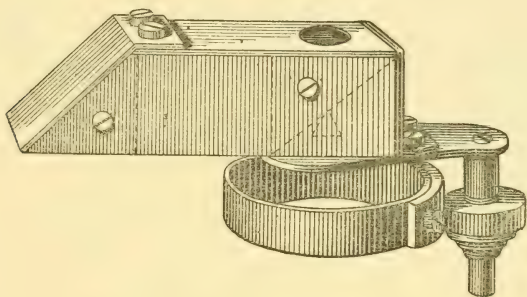


Fig. 21.—Nachet's Grapho-Prism.

In the freehand production of a microscopical drawing one must be skilled in drawing. The necessity of this exceptional artistic skill has led some of the expert microscopic artists to devise an instrument which greatly facilitates the work and renders it comparatively easy to any microscopist. This valuable instrument is the grapho-prism. In drawing a picture from a specimen under the microscope, all depends upon the optical measurements. If the object be so small that the combined compound microscope is brought into use, the optical measurements are easily and simply procured by means of a micrometer-eyepiece ; but if the object be an extremely minute one (such as germs, bacilli, microbes, etc.), other means must be employed to satisfy this necessity, and opticians have therefore manufactured micrometer objectives which, of course, are divided into the one-thousandth part of a millimetre : but with the oil-immersions the lines of the graduated millimetre-scale are invisible, therefore inapplicable.

If only an artistic (impressional) reproduction is required, it may generally be produced by freehand without the aid of any accessories, since in such a case the mathematical correctness is not so necessary as in a drawing made for scientific study, where all depends upon its mathematical correctness if it is to be instructive, and under the latter instances the optical measurements are the first consideration and of paramount importance.

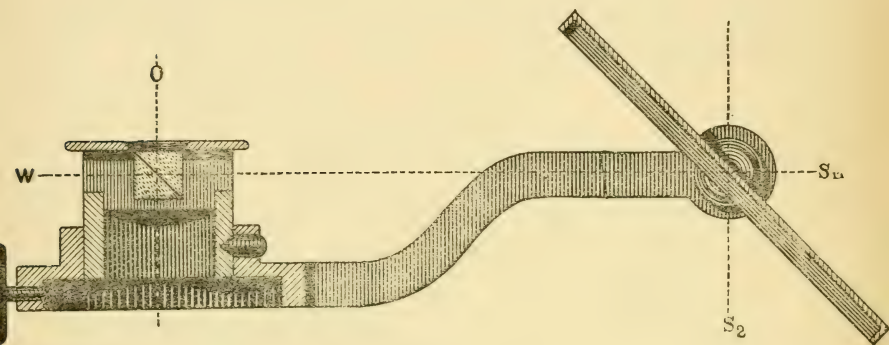


Fig. 22. —Abbé's Grapho-Prism.

The most practical and most frequently applied drawing-apparatus in microscopical work is the Camera Lucida. This and all similar instruments work upon the same principle—that is, the object and the paper are seen with the one eye, while at the same time the picture is reflected into the eye by means of the mirror or prism. As the picture is seen upon the paper beside the microscope, its contour can easily be reproduced upon the paper with the point of a pencil. Thus a fac-simile of the utmost mathematical and scientific correctness and exactness may readily be produced. He who has by practice learned to look into the microscope with one eye and to hold the other eye open at the same time may succeed even without the use of a Camera Lucida. If he gazes with one eye into the microscope and with the other eye at a piece of paper lying beside it, in a few moments the observer will find the object projected upon the paper, and will thus be able to sketch the outlines of the image with comparative ease and exactness.

In the execution of the drawing of a microscopic object it is best to use good drawing paper or Bristol board, which should be either pale yellow, pale green, or white, slightly tinted. It is also advisable to have the paper fastened upon a smooth board. First use a soft and finely sharpened black-lead pencil, in order to secure the outlines and the contour of the picture. It should be slightly shaded without pressure, then with bread crumbs erase most of it again ; after that with a harder and finely pointed pencil retrace the outlines of the first drawing, again using the prism for comparison in order to make any necessary improvements, and to secure perfect accuracy ; at this moment is the proper time to do the shading, if such is required, and this can easily be done with the point of a pencil and an eraser, or still better with charcoal and a soft cloth. For the execution of a coloured drawing in which a variety of colours are used, water colours are most commonly used, and are to be preferred, but coloured pencils, and even oil colours and pastel crayons may be employed instead. I wish here to call especial attention to the fact that in shading it is advisable to shade off the uncoloured parts first with black, of course, particular care being taken that the shading does not extend into the coloured field. This is necessary to avoid confusion and to preserve scientific accuracy. It is also decidedly recommended to use a variety of colours, especially so in the drawing of very minute objects, such as endothelium, and epithelium-cells, fibrous and connective tissue-cells, blood and lymphoid-cells, etc. Also in the drawing of a whole slide (specimen), or only a part of it, it is almost an absolute necessity to use a variety of colours. The contrasting colours will not only make a drawing or an illustration more elaborate and intelligent, but decidedly more comprehensive and instructive.

Let me here refer to the pertinent and emphatic declarations of Prof. Virchow, one of the most noted and expert pathologists of the nineteenth century, who said that he would not give "ein pfennig" for illustrations, drawings, or sketches that were not correct or exact, because they would invariably convey a false impression. He further declares that all lectures, demonstrations, original articles, or manuscripts of any kind, must be

accompanied by first-class drawings, sketches, illustrations, etc., if they are to be considered bona fide, and which should be strictly a *chef d'oeuvre*. I would therefore advise every practical and expert microscopist, and particularly those who are not skilled in drawing or sketching, and in the art of producing microscopic illustrations, to make use of the Grapho-prism. Especially would I advise students of practical histology, physiology, pathology, pathological anatomy, bacteriology, embryology, pharmacology, etc., to use the Grapho-prism hand in hand with the microscope. If a student produces a careful drawing of the object under microscopical consideration and examination, he will certainly comprehend the subject more readily and fully than would otherwise be possible.

This method of using the microscope and Grapho-prism combined together, I denominate MICROSCOPY DUPLEX, in contradistinction to the other mode of studying microscopy in its simple form without the Grapho-prism, MICROSCOPY SIMPLEX.

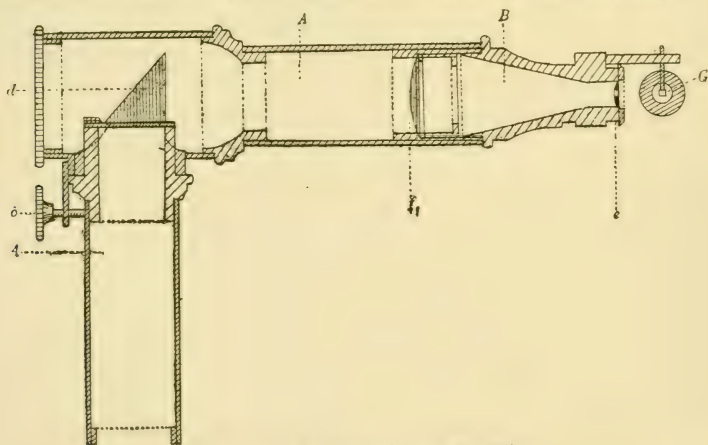


Fig. 23.—Oberhauser's Grapho-Prism.

I will now make a few explanatory remarks concerning the Camera of Oberhauser (Fig. 23), which is somewhat more complicated than the others; for this reason I will give more particularly the details in regard to its practical application in microscopy duplex. The ascending rays from the objective are totally reflected through the large prism (*d*) into the horizontal arm (*A*).

If the ocular is placed in a horizontal position (*B*) it directs and throws the rays into the small prism (*G*), at an angle of 45° , providing it is focussed from the right position, where it is again reflected at a right angle into the eye of the observer. Oberhauser's Camera is liked for the reason that it does not create a disturbance nor a confusion by the reflection of the projected picture at a right angle upon the projected paper placed in a horizontal position. The Oberhauser Camera is attached to the tube of the microscope at the ocular end without any trouble or loss of time, and would be considered perfect but for one deficiency. When the microscopic picture is twice reflected it loses a considerable portion of its clearness and accuracy; that is, in its clearness and exactness. This is especially the case when high powers, oil immersions, etc., are used; it is then only by the most concentrated light that the special and superficial contour of the microscopic picture can be procured.

Starches.

BY JAMES W. GATEHOUSE, F.I.C.

THE importance of starch will be at once perceived when it is considered that starch-containing foods form the staple nourishment of the human race and of domestic animals, there being scarcely an order, and if we except certain fungi and algæ, barely a species in the vegetable kingdom but contains starch in some form or other during certain periods of its growth. Starch is not only found in plants, but in animals. Stafford found starch in the blood of epileptic patients. Virchow found amyloid matter in the spinal cord and nerve centres, and traced its distinct connection with diseases of the bones.

Whether we obtain starch from ears of wheat, tubers of the potato, the thallus of lichens, from the pith of the palm, or from the tissues of animals, it invariably consists of the same chemical elements—carbon, hydrogen, and oxygen, united always in the same proportions; the hydrogen and oxygen being in the proportion to form water, whilst the number of carbon atoms is one less

than the number of water molecules, the chemical constitution being usually expressed by the symbol, $C_6 H_{10} O_5$, or a probable isomer $C_{18} H_{30} O_{15}$.

Starch, when heated to $158^{\circ} F.$, swells up and forms a paste, from which alcohol precipitates a white powder consisting of soluble starch. Heat alone dries the granules, and at a temperature of $320^{\circ} F.$ converts them into a soluble modification called dextrine. This dextrine differs from starch in being soluble in cold water, and in producing a reddish brown colour with iodine instead of the bluish purple so characteristic of true starch. This test, above all others the most delicate for starch, can most readily be applied under the microscope, all that is required being to place a small portion of the material to be examined on a slide with water under a covering glass, and by means of a delicate pipette introduce a solution of iodine in potassium iodide, to the edge of the cover. A slip of bibulous paper now placed on the opposite edge will, by capillary attraction, draw out the water from under the cover, and cause the iodine to run in gradually, tinting any starch granules blue, the colour gradually deepening as the action of the re-agent is the more prolonged. Dextrine, under similar circumstances, takes a copper colour. Inulin, however (a variety of starch to be obtained from the tubers of certain *Compositæ*, as dahlia and elecampane) is not thus affected by iodine.

The composition of starch cannot be considered alone, for during the growth of a plant we find in it certain other constituents having the same composition as starch. Of these, dextrine, gum, and sugar need only be mentioned here as connected with our subject, as we shall find it probable that in the life of the plant these three substances, or others analogous to them, are the immediate principles from which starch granules are produced. In this connection I may mention that starch boiled with dilute acid loses its power of becoming blue with iodine, and is, in point of fact, first of all converted into dextrine, and ultimately into glucose, a form of sugar. This same change is brought about naturally in the plant by the action of diastase.

Having spoken thus far on the chemistry of starch, we will next proceed to investigate its physical characters, and herein not a step could be taken without the aid of the microscope, which,

indeed, in conjunction with the chemical tests above referred to, is all in all in the determination of the kind of starch, or source from which any variety of starch may have been obtained. Looking at any sample of starch, we perceive it under the microscope to consist of more or less rounded particles, which, if moist, will be seen to be marked with certain concentric rings surrounding a spot called the hilum. Old observers considered these granules in the light of cells, the rings being markings on the cell wall, whilst the hilum represented the point of attachment to the wall of the primary cell wherein they were enclosed.

A section of any tuber or starch-containing seed before germination—such as the pea—will show the fallacy of this; the granules, though certainly contained in cells the same as any other cell contents, are not attached either to the cell-wall or to each other, but appear to be merely deposited therein, and to act as a reserve store for the future formation of cells. Thus, although the cells in a pea are full of starch before germination, yet let germination once ensue, after some two or three days iodine no longer produces the magnificent blue colour indicative of starch, but the browner tint showing the transformation into dextrine, and after from ten days to a fortnight no trace of starch granules are visible, all having been dissolved by the diastase, and left the cells wherein they were deposited to produce cells in the young shoots, before the formation of roots enables nourishment to be extracted from the soil. This may be beautifully illustrated by observing two sections of pea under the microscope, one being cut before germination; the other ten days after.

How, then, are starch-grains produced? From what are they formed? Do they grow in layers, as would seem to be indicated by the markings? and if so, are these layers deposited from within outwards, as would be the more natural supposition, or in the opposite direction? Many observers have worked on this subject, amongst whom I may mention Raspail, Fritzsche, Buck, Allman, Crüger, Schlieden, Virchow, Raine, Nagelli, and Sachs. These are only a few of the names of workers in this subject who have attempted to elucidate the growth of the starch granule, nearly every observer coming to a slightly different conclusion to others.

The observers on the subject may, however, be divided into

three classes :—(1) Those who considered the granule to be composed of layers rolled, so to speak, over each other.

(2) Those who considered it to be a vesicle increasing by inward growth, the vesicle continually expanding up to a certain point to allow of further internal increase. On this supposition the internal layers would be the younger and the external the elder. This view was strongly supported by Allman, as he found the internal portion more easily attacked by such re-agents as sulphuric acid, acetic acid, and iodine, than the external.

(3) Schlieden and Crüger, however, from identical experiments connected with observations on compound starch granules, which contained two or three hilums or centres of growth, came to the opposite conclusion—namely, that the external layers were deposited later than the internal, all with the exception of the nucleus being chemically identical.

Nagelli was of opinion that starch grains are utricles, consisting of a membrane and fluid contents, concentric layers being deposited on the inside of the membrane as in lignifying cells; the cavity of the utricle, the so-called “nucleus” or “hilum,” thus becoming reduced to a most minute excavation always filled with fluid. When starch is first treated with iodine, and then immediately with sulphuric acid, the granule swells up and the lamellæ generally separate from each other. This experiment may be made by any one possessing a microscope.

From the latest observations of Sach and others, starch would appear to be actually produced by the deposition or rather precipitation of minute starch particles, which, cohering to each other, gradually form the granule, the growth of the grain being produced entirely by intussusception—*i.e.*, by the intercalation of new particles amongst those already deposited. This mode of growth necessarily depends on the permeability of all parts of the grain to the aqueous solutions, from which starch particles are precipitated. This again can only be explained by supposing the starch substance to be discontinuous, consisting of minute, invisible particles, each of which possesses the power of attracting moisture and enveloping itself with an aqueous envelope.

Where the particles are large, the number of these aqueous envelopes in a given bulk of starch will be less than when the

particles are smaller, and the markings observed in the granule, together with the hilum or nucleus, are caused by the greater or less amounts of water, and therefore alteration of refractive power in various parts of the granule. The stratification of a starch-grain disappears when the water is wholly removed either by drying or by chemical re-agents, or when it is rendered equally aqueous in all its parts. This abstraction of water may be accomplished by the action of absolute alcohol, whilst the absorption of water may be effected by treatment with dilute solution of potash.

Starch-granules, although rendered blue in all their parts by iodine, yet consist of two distinct modifications—granulose, which is the more soluble and readily acted on by iodine, and starch cellulose, which is not so readily acted on. This latter is present only in the proportion of from two to six per cent. Granulose may be extracted either by saliva or by a saturated solution of salt, containing one per cent. of hydrochloric acid.

It is not absolutely known what are the principles in the plant from which starch is precipitated, but here an observation of Rainey's offers a valuable suggestion. He found that a mixture of gum and dextrine, whether in acid or alkaline solutions, caused a precipitation of true starch particles. The best way to observe it under the microscope is to acidify a solution of dextrine with citric acid, and under the microscope to treat first with gum and then with iodine, or the iodine may be added first and the gum after; in either case, colourless starch corpuscles are first formed, turning blue as they unite with the iodine. Now, as we know that gum, dextrine, or their isomers are always present in living plants, it is but reasonable to suppose—although the fact has not been actually demonstrated—that these substances play an important part in starch formation; we should thus find in the plant a gradual transformation of starch into dextrine, sugar, and gum, etc.; and again the re-formation of these bodies into starch to act as a reserve food in the process of further cell formation.

With respect to this portion of our subject, it is found that starch is not usually produced in the living plant except in presence of chlorophyll. In a perfectly dark place, where green chlorophyll cannot be formed, there starch is not usually produced, and if starch, which has been formed under the action of chloro-

phyll in the light, be placed in the dark, it is again absorbed by the living plant as seen in Spirogyra, etc. Although this is the general rule, yet some tuberous plants—as the potato, where the tubers contain an abundance of starch—seem to possess the property whilst germinating of absorbing the starch of the tuber and again depositing it, although no ray of light has ever reached the plant, thus trying to put forth its powers. In perfectly dark cellars it is not uncommon to find small young potatoes from the size of a pea to that of a walnut, when the tubers have been left to germinate. The shoots here are wonderfully elongated and quite white from want of light and of the formation of green chlorophyll. As the young tubers thus formed are always small and few in number, it is very doubtful whether any further development of starch has really taken place, but it is thus certain that after the starch of the tuber has been rendered soluble it may be again deposited at least partially in absence of chlorophyll and of light.

As starch enters so largely into the food of man, it frequently becomes necessary to distinguish between the starches, as derived from various sources, in order to be able to state whether any admixture may have been added to a given article. Thus, it sometimes occurs that wheat-starch finds its way into pepper, potato-starch partially replaces the more expensive arrowroot, and rice has been made up to resemble sago or tapioca.

These adulterations can only be discovered by examining the intimate structure of the article by the microscope, and in order to do this it is necessary to note—(1) Shape and size of granule. (2) Position and shape of hilum. (3) Position and clearness of concentric rings. (4) Facility with which the granules polarise. Granules of starch vary in size in different samples, from 1-5,000th of an inch—as in liquorice, ipecacuanha, and rice—to the 1-200th of an inch in *tous les mois*. Now, although from the natural growth of starch-grains we may find in any sample of a large variety a number of very small grains, yet on the whole the size may be fairly relied on; at least to the extent that in each species the size of the grains will never be larger than a certain maximum.

In this microscopic examination of starches we may divide them into certain groups:—

I.—Oval, more or less, and with hilum and rings well visible.

Amongst these we have—*Tous les mois*, 1'250 (2); arrowroot, '00148 ('8); Natal (rings very plain) potato, '0025 (1'5); turmeric, '00148.

II.—Oval or round hilum and rings visible with difficulty. Wheat, '00185 (1); barley, '00073 ('4); rye, liquorice, '00018 (1); jalap, '00185 (1), polarizes brightly; chestnut, '0009 ('5).

III.—Hilum well developed, rings not well seen. Bean, '00135 ('75); Calabar bean, '003 (1'6); pea, '00135; maize, '00074 ('4); polygonal.

IV.—More or less truncated. Sago, '002 (1'2); tapioca, '00074 ('4); arum, '00056 ('3); colchicum, '00074 ('4); podophyllin, '0004 ('25).

V.—Very small and generally polygonal. Oat, rice, pepper, ipecacuanha.

The figures in brackets refer to the size of the granule as compared with that of wheat-starch. I have attempted to identify and separate the granules of mixed starches by other means. Taking their specific gravities seemed to present a probable solution of this problem. This, however, failed practically owing to the very slight difference between the specific gravities of various starches.

Another method tried was to sift the granules from each other by means of fine sieves, composed of a layer of cellular tissue attached to the end of a glass tube about one inch long and one-fourth of an inch or less in diameter. If layers of cellular tissue, such as the inner cuticle of leaves, could be found, in which the size of the cells varied as the starch granules, it would be quite possible to separate small from large granules exactly as different sized stones may thus be separated from each other by means of stronger sieves.

A BALLOON, intended to make headway against air-currents of twenty-eight miles an hour, is being made in France. It will be similar in form to the *La France* of 1884—85, but larger—two hundred and thirty feet in length and forty-three feet in its greatest diameter. It will weigh sixty-six pounds per horse-power, and will be propelled by a screw in front, with a rudder behind.

On the Cultivation of Diatoms by Artificial Means.

BY DR. MIGUEL.

Translated from *Le Diatomiste*.

Plate VIII.

Part II.—ON THE ARTIFICIAL GROWTH OF MARINE DIATOMS.

IF the experimenter is near the sea, he will use natural sea-water for the growth of Marine Diatoms ; but if he has at hand only soft, or river water, he must add the undermentioned salts in the following proportions :—

Soft Water (rain or distilled)	...	1000 c.
Common Salt (Na. Cl.)	...	25 grms.
Sulphate Magnesia	...	2 grms.
Chloride Magnesium	...	4 grms.

This artificial sea-water, which, like the natural water of the sea, may perhaps be somewhat modified as to its composition, readily lends itself to the growth of Diatoms. In my comparative experiments this compound has often given finer growths than the natural sea-water, and sometimes the reverse has been the case. But whether it be natural or artificial sea-water, there is in them little of nutriment for the Diatoms, whose behaviour in this respect is similar to that of Fresh-water Diatoms. It is, therefore, necessary to add to them the solutions described as *A* and *B*,* and also organic substances in suitable quantities, as bran, straw, or some ribands of the yastera, commonly called “vraick.”

In some cases, in order to obtain precise comparative results, you should make, two or three weeks in advance, a nutritive maceration, filtered ; and also a fluid sufficiently charged with nitrogenous substances. In all cases sterilisation must be effected at 70°C. But you must be very careful how you add to the sea-water gelatinous lichen or other marine plants (either fresh or dried) from muddy localities. In a word, if you wish to imitate what takes place in Nature, and you yield to this desire, you will obtain such a putrescent medium, that in twenty-four hours the Diatoms that you have sown will be irremediably destroyed. In

* See page 37.

these delicate cultivations, the experimenter will never regret that he has used the greatest parsimony in adding organic substances.

I have before said how these sowings should be made, so that I need not return to that part of the subject; but I ought to tell how you may, and ought, to procure Marine Diatoms, for charging these solutions.

By immersing fresh oyster shells that have been very carefully deprived of all traces of the flesh of the mollusc, so as to avoid all putrefaction, in sea-water, either natural or artificial, you form a growth that can be carried on for one or two months, and produce often fine specimens of Diatoms, that you can further cultivate at leisure. The oysters should be placed in stone-ware or earthen vessels, which by their opacity scatter the reflections of the sun and walls, and only allow the light that proceeds from near the zenith to reach the Diatoms.

If a correspondent is called on to send away Diatoms, he should, in the first place, separate by decantation all muddy substances, and after washing them with clean sea-water five or six times, enclose them in a tight vessel with a large excess of sea-water. By adopting this plan, living marine Diatoms may be kept several weeks, whilst if Diatoms be sent with the sediment from which they have been gathered, putrefaction will kill them in a few days.

When the growth of marine species has to be continued for a long time, it is indispensable to prevent the sea-water from altering its density, etc., by concentration. Many forms of apparatus have been proposed for preventing the evaporation of the water, and the lowering of the level which results from it; that which I represent in Fig. 1, Pl. VIII., appears to me the most practicable and trustworthy.

Fig. 1.—*V* is a vessel containing growing Diatoms, filled with sea-water. *F* is a flask containing distilled water, to maintain a constant level. *S'* is a siphon which conducts the water, and *S* a tube that permits the action of the siphon when the level of the liquid in *V* falls below its orifice, and when, consequently, the air in the flask communicates freely with the external air. The theory of this intermittent siphon is too simple to require any explanation to my readers.

Part III.—GENERAL DIRECTIONS AND NOTES ON THE GROWTH OF DIATOMS.

DESPITE all the precautions that I have been obliged to insist on, the Diatomist will hug a delusion if he thinks that the cultivation of the frustular algæ is always an easy task. In order to avoid the annoyance that failure occasions, the operator ought, in the first place, to make serious study of the different manipulations that the cultivation requires. Then he should understand thoroughly the light that visits his laboratory, and which will often be found to act in very different manners, as the days are long or short. He must take precautions to protect his growths from the excess of the luminous radiations, as well as from the deficiency of these radiations. Some screens, some curtains, some stages, so placed as to admit of the governing of the lighting, which ought to be continually looked after and intelligently managed, will be required. On the dark and rainy days of summer, the growths of the large and beautiful Diatoms should be exposed to the north, and immediately behind the sashes ; if the sun burns, they should be carried into a half-darkened chamber. In winter, constant exposure towards the north offers less difficulty, and the oversight is much easier.

Thus, then, in the majority of cases, we must protect the Diatoms against too much light. If in Nature the Diatoms instinctively fly from these luminous rays, that are hurtful to them, and seek those that are helpful in the narrow part of the flask, they are evidently compelled to submit to such physical conditions as the experimenter forces on them ; if these conditions are adverse, the Diatoms will not develop, and this is a frequent cause of failure. On the other hand, if many species are mingled together, those that receive the luminous radiations most congenial to them which develop, will occupy the centre, and smother the adjoining species. You will quickly perceive many kinds of little Nitzschies displaying their pretty frustules, after the manner of weeds ; if they have once got foothold in a maceration, it is difficult to get rid of them, whence the necessity, as I have before said, of establishing for each kind of Diatom a special form of culture, suitable for assuring the predominance, and favouring the multiplication of the species desired.

It often happens that in the rough sowings you may introduce into the liquids with the Diatoms algæ, or spores of algæ, of protococci, desmids, confervas, etc. ; more rarely in the liquids I have described, cryptogams of the moss tribe increase very annoyingly, but the green algæ are the most troublesome, and if you cannot eliminate them, they will pervade the whole liquid, taking up rapidly the organic and mineral substances contained in the maceration, and the life of the Diatoms will be rendered impossible.

If the Infusoria (properly so called) are of but little injury to the Diatoms, we cannot but consider as dangerous some of the Paramecæ, which will swallow as they pass, in their gluttony, a little navicule, a cyclostella, or an achanthe ; nor can we say otherwise of a class of Protozoons—the Rhizopods—which, like the Vampyrellas, destroy them one by one, and spoil the growth. I have in my laboratory a kind of Actinophrys, which, sown along with the Diatoms, in a maceration highly nutritive for siliceous algæ, disorganised and killed them by enclosing them for a long time in its protoplasmic mass. The Glass Worm, and many other annelides, also spoil the growth of Diatoms, but it is easy to get rid of them by killing them.

The Bacteria, as previously said, also attack the Algæ that we are growing, and it is not rare, even in the most successful growths, to distinguish moveable frustules perfectly endochromed, literally covered with the filaments of Bacteria, fixed perpendicularly on the thallus, giving them the appearance of cells bristling with pseudopodia. When the medium is very favourable to the production of schizomycetes, all the Diatoms are tainted with the bacillus disease ; they end by becoming immovable, their valves open out, and then parasites of all sorts settle down on their endochrome, which is destroyed in a few days ; and the Protozoa make only a few mouthfuls of this protoplasm which has become accessible to them, and in a very little time the Diatoms are entirely ground up.

Without being directly attacked, Diatoms are also pathologically influenced by the proximity of many cryptogams. I know one moss that, grown by the side of these algæ, induces among them a kind of “melanose,” or “anthracnose,” manifesting itself

by the gradual blackening of the protoplasm of the cellules, at the end of the disease the contents of the frustules becomes grey, but in the dead cellules the shrivelled endochrome becomes as black as coal. The chapter relating to the diseases of Diatoms under artificial culture is therefore very extensive, and the diatomist must expect numerous infections, very varied in their manifestation, and which may come altogether unforeseen, to neutralise his operations.

Hitherto we have only spoken of the cultivation of Diatoms by daylight, which is uneven and variable in its action. To avoid the irregularities of the natural light, I have tried, and succeeded, in growing these algæ by gas-light. The flame of a jet burning from 50 to 100 cm. of gas per hour, is sufficiently life-giving to promote, at a distance of 0·20 cm., the growth of the greater part of the marine and fresh-water Diatoms. You can even obtain splendid growths of *Melosira* and *Fragillaria*, which fill all the liquid, giving magnificent tufts, formed by an infinity of yellow filaments, perfectly endochromed. Nearly all the marine and fresh-water Diatoms, as I have said, develop under the action of gas-light, and I have no want of success to notice, even in the growth of the marine *Pleurosigmas*, the cultivation of which is a most delicate affair.

When the artificial light is feeble, as when 40 to 50 litres of gas are burnt in an hour, many of the Diatoms that are mobile in daylight increase and multiply, without sensible movement, but as soon as, and in proportion as the light is increased, they are seen to regain their habitual movements, even when their length is greatest, as certain *Naviculas*, *Pleurosigma Balticum*, *Cymatopleura solea*, the long *Synedras*, etc.

The apparatus that M. Admet, of Paris, has constructed for me, for the growth at the same time of a great number of Diatoms, is represented in Pl. VIII., Fig. 2. It is composed essentially of a cylindrical chamber, capped with a conical cover, *E*, surmounted by a chimney that draws off the products of combustion. The vertical division and the lower part of the chamber are furnished with a double envelope, that allows a current of cold water to circulate around them, and thereby to lower the temperature of the growths when they are acted on by very intense luminous rays.

For lighting the growths a bat's-wing burner, or a circular burner, may be used, entering the chamber by a central aperture, and of which the supply of gas is regulated by a pressure guage, and the quantity burnt per day being ascertained by a small meter. You can also place between the growths and the light cylinders of glass, coloured or ground, so as to diminish the intensity of the luminous and heating rays; rendering the flame of the gas less flickering, more brilliant, and modifying the nature of its radiations. The electric light, less heating and more easy to manage, might certainly be substituted for gas-light with advantage, but I have not been able to employ it in my experiments.

In order not to confuse the description of the simple facts that I have noticed, I have not spoken of the many modes of growth that the operator may attempt. Not only is it possible in certain cases to employ glass vessels, either plain or coloured, stoneware, or earthenware, but to carry on the cultivation in continuous currents of water, provided that the water employed to renew that which the Diatoms have exhausted for their nutrition shall arrive slowly, and after having been perfectly filtered. To carry out this arrangement, the filtered water should be conducted to the centre of the liquid, and delivered, drop by drop, from a glass tube, or a fine thread, whilst the excess is allowed to escape at a lateral tubule at the top of the glass.

You may also introduce into the nutritive liquids some substratum on which certain Diatoms love to fix themselves. Twigs or bits of wood that have been previously boiled, or macerated for a long time previously; fragments of marble, of earthenware, of chalk, of various rocks, oyster-shells, flint nodules, etc. The introduction into the growths of these various substances, that are more or less indestructible, generally without action on the development of the Diatoms, permits them to seek and choose for themselves the spot most favourable to their multiplication. To cultivate some very fragile kinds I have prepared deposits of artificial flocculi, in the middle of which many Diatoms find the degree of light that is most favourable to them. These flocculi are composed of the silicate of magnesia, hydrate of alumina, or of pure hydrate of silica. The two first substances form flocculous clouds, of which the extremely slow aggregation permits the

algæ to penetrate the deposit, there to remain, and thence to issue when circumstances require it.

All these details, which are important, will better find their place in the description of the special arrangements required for the cultivation of each kind of Diatom, for there is only a small group of species that accommodate themselves easily to the same physico-chemical conditions.

If, for example, we desire to obtain an abundance of the little common *Nitzschia*, which is found in almost every place, you should add to the maceration some decigrammes of gelatinous silica, produced from mineral salts, and expose them to a strong light. Under these conditions, there are scarcely any species, but some *Cyclotellas* and *Synedras*, which can multiply in the same. If you wish to establish the predominance of *Cyclotellas*, it will be necessary to add to the liquid 4, 5, or even 10 per 1,000 of chloride of sodium ; if, on the contrary, you wish to prevent their multiplication, you should substitute for the chloride of sodium chloride of calcium, and the *Nitzschias* will become predominant. The *Pleurosigma attenuatum* fears not the light ; the *Pleurosigma sculptum* high temperatures ; *Pleurosigma angulatum quadratum* and *Pleurosigma Balticum* become superb in yellow light, etc. etc. From this you can see that no one mode of culture is equally applicable to all species. If the nutritive substances, organic or mineral, are few, the proportions in which they ought to be employed, according as you wish to obtain a great quantity of this or that species of Diatoms, is very different.

Thus experimenters need to establish with certainty the chemical composition of the macerations best adapted to the development of those Diatoms that they desire to study, and to determine with exactitude the physical conditions (light and heat) for which they have a marked predilection. This work is considerable, long, and delicate, but its utility is undoubted ; for by means of the artificial culture of Diatoms, we shall, I believe, arrive at an elucidation of the many obscure points that surround their history. With a little patience and perseverance, you will be able to cultivate all kinds easily ; for my part, those that I have myself procured, or that I owe to the kindness of M.

FIG 1

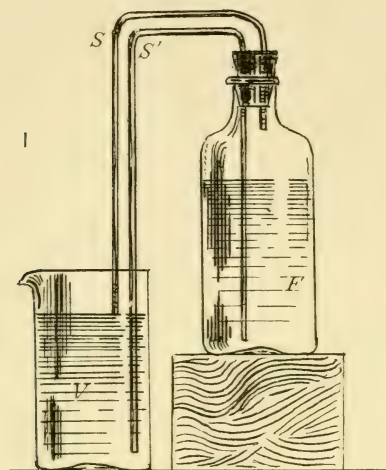
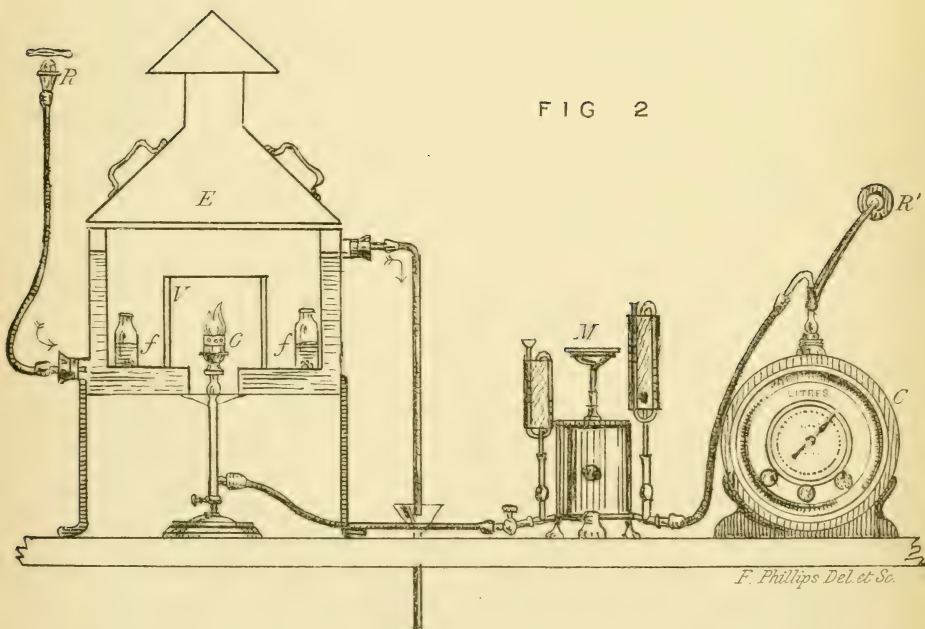


FIG 2



Apparatus for the Artificial Cultivation of Diatoms.

Tempère, have shown themselves capable of increasing in the macerations that I have prepared ; one only, the *Rhizosolenia gracellenia* that M. Bergon has obtained from a gathering recently made at Trepars, has shown itself refractory ; but I ought to add that this want of success is not without a cause, for these *Rhizosolenas* arrived in an advanced stage of putrefaction, with an endochrome completely granulated ; whilst the numerous *Bidulphias* which were found mingled with them, less affected by the poisonous gas resulting from the decomposing matters, increased and multiplied without difficulty.

EXPLANATION OF PLATE VIII.

- Fig. 1.—*F.*, Flask containing distilled water ; *V.*, Cultivating vessel ; *S'*, Siphon conveying distilled water into *V.* ; *S.*, Tube which permits the siphon to act.
- „ 2.—*E.*, Dark Chamber for cultivating the Diatoms ; *f.f.*, Cultures ; *V.*, Glass cylinders ; *G.*, Gas-burner ; *R.*, Water-tap ; *R'*, Gas-tap ; *C.*, Indicator ; *M.*, Pressure-regulator.

The Bot-Fly of Man.

A WRITER in *Insect Life* for September states that in Honduras and other Central American Countries there is a fly that deposits its ova in the skin of human beings. The naked Indians have a few, but the whites, who wear shirts, have ten times as many. Mr. David Logan, now in Massachusetts, passed about twenty years of his life in tropical forests hunting for mahogany, and has had at least a hundred of these parasites in different parts of the body at the same time. On one occasion he had eighteen of the maggots squeezed out of his back. The back and shoulders are especially subject to the attacks, although they are not limited to those parts. Mr. Logan was once attacked in the upper lip.

The first evidence of the presence of the larva in the skin is the appearance of a small furuncle, not painful, but giving the victim a sensation of uneasiness. Close inspection shows that there is a minute orifice in the middle of the swelling. When first detected the larva is of about the size of a pin's head. If not dislodged for a period of five or six weeks, the grub will attain to the length of an inch. The treatment employed by the natives is to cover the infested parts with a piece of tobacco leaf just over the perforation of the integument, and soon afterwards the maggot can be forced out. It is probable that the species concerned is the *Dermatobia noxialis*, commonly known to Spanish Americans as *Ver-macaque*.

An Improved Means of obtaining Critical Illumination for the Microscope.

BY HENRY G. PIFFARD, M.D.

CRITICAL illumination is that sort or kind of illumination which best conduces to the revelation of the intimate structure of microscopic objects. The illumination is said to be "critical" when the image of the radiant (lamp-flame or other source of illumination) is brought to a focus by mirror or condenser at the plane of the object under examination. Skilled microscopists are pretty well agreed that the most convenient and feasible means of obtaining critical illumination is by focussing the edge (not the flat side) of a half-inch kerosene flame on the object.

The writer is unable to use with comfort either gas or an oil lamp in microscopic work, but has found that he can work by electric light for several hours continuously without inconvenience. Attempts to obtain satisfactory critical illumination from this source have occupied a portion of his time during the past two years. Without referring to the devices he has abandoned, he will simply describe the one at which his experiments have terminated, leaving to others the opportunity to still further improve it.

The ordinary electric lamp in domestic use has an illuminating value of sixteen candles, and its thread-like filament is brought to incandescence by a current having an intensity of about half an ampère under a pressure (in the Edison system) of from one hundred and fifteen to one hundred and twenty volts. The light is distributed over a filament about five inches in length. Such a light is not a desirable one for microscopic work. It would be much better to have the light more condensed by the use of a shorter and thicker filament. There is no difficulty whatever in constructing such a lamp, but if it were brought directly into the Edison circuit, its life would be exceedingly brief; in other words, a certain length of filament is required to withstand the pressure of the current from the main. Two things, then, are needed: First, a lamp with a short and thick filament;

and, second, a means of properly connecting it with the street service.

Having decided on the character and form of lamp desired, I applied to the Edison Lamp Works to have it constructed according to the plans and specifications which I furnished. These were carried out as requested, and the result was a lamp of fifteen-candle power, requiring a current of about three amperes under a pressure of fifteen volts.

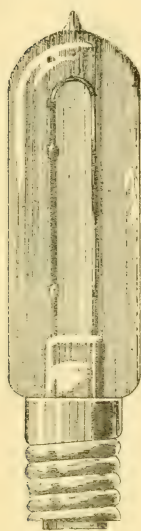


FIG. 24.
The author's electric illuminator.

The lamp in question possessed certain peculiarities of construction, as will be seen by an examination of the cut (Fig. 24).

The glass bulb, instead of possessing the pear-shaped form usually met with, is cylindrical, and about three inches in length by an inch in diameter. At first glance the carbon filament would appear to have the ordinary horse-shoe form, and to be of the usual length (four to five inches). A closer inspection, however, shows that the carbon is actually but three quarters of an inch in length, while the rest of the apparent filament is composed of copper wire, arranged so as to hold and support the carbon in a vertical position. It will also be noticed that the carbon is much broader and thicker than in the ordinary domestic electric lamp. When this carbon is rendered incandescent by the passage of a suitable electric current, we will have, when the lamp is in position, a vertical streak of light, of intense brilli-

ance, about three quarters of an inch long, and apparently an eighth of an inch wide. The minified image of this is focussed by mirror or condenser on the object we desire to examine, and constitutes "critical" illumination. If now we proceed to the examination of the object, with, for instance, a quarter of an inch objective, we observe that the field is not evenly illuminated, but, instead, a central brilliant streak, on each side of which the light is comparatively feeble. The portion of the object within the area is now illuminated in the manner most favourable for the revelation of its intimate structure. In systematic work, critical

illumination is rarely called for, except as a means of control, and subcritical or diffuse illumination, as obtained by racking the condenser a little out of focus, is preferable and more commonly employed. The lamp here described furnishes a light for ordinary work, which, in many respects, is preferable to any I have heretofore employed.

While this fifteen-volt lamp can be readily maintained at full incandescence by the current from an eight-cell storage-battery, the care of this latter is by no means an insignificant matter; and I am not prepared to recommend its use unless one has access to one of the street circuits. In this city we have at our disposal either the Edison circuit, with a pressure of from one hundred and ten to one hundred and twenty volts, or the alternating current, distributed to houses under a pressure of fifty-five to sixty volts. If the fifteen-volt lamp be connected directly with either of these circuits, it would be instantly destroyed. It is necessary to neutralise, or take up a portion of this pressure, by the introduction of a suitable resistance. This can be conveniently accomplished on the Edison circuit by the interposition of a one-hundred candle power, one hundred volt, three ampère lamp of the "*municipal*" type, the two lamps being connected in *series*. Both lamps will, when thus arranged, burn at full incandescence; but, as we do not desire to employ the larger lamp, this may be placed under the table and covered with a box.

In photo-micrography the writer has made use of nearly all the methods of artificial illumination that have been proposed, including the electric arc, electric incandescent with coiled carbon of one hundred candle power, calcium light, Welsbach gaslight, and kerosene oil. The lamp here described he finds infinitely more convenient and amply efficient. For the study of absorption spectra by means of artificial light, this lamp gives an ideal illumination.—*New York Medical Journal*.

Dr. A. Famintzine has found some remarkable new forms of bacteria in the aquarium of the Botanical Laboratory of the Imperial Academy at St. Petersburg.



ZACCHARIAS JANSSEN,

INVENTOR OF THE MICROSCOPE.

Fac-simile.

After P. BORELLUS, DE VERO TELESCOPII INVENTORE.

The Microscope: its Construction and Management.*

MR. WYNNE E. BAXTER has favoured English-speaking microscopists with a carefully translated edition of Dr. Henri Van Heurck's treatise on the Construction and Management of the Microscope, including Microscopical Technique and Photo-micrography. The volume before us is a very handsome one, being printed on stout Imperial 8vo. paper, with broad margins. Although it is called a translation of the Fourth French, it may really be considered as a fifth edition, so large an amount of new and interesting matter has been added.

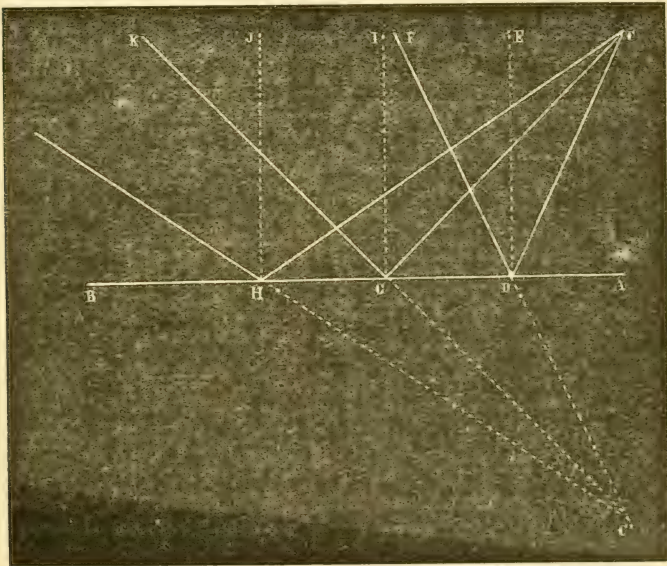


Fig. 25.

* "The Microscope: its Construction and Management," including Technique, Photo-Micrography, and the Past and Future of the Microscope. By DR. HENRI VAN HEURCK, Prof. of Botany at the Antwerp Botanical Gardens; Late Pres. Belgian Micro. Soc.; Hon. F.R.M.S. and New York M.S. English Edition, augmented by the Author from the Fourth French Edition, and translated by Wynne E. Baxter, F.R.M.S., F.G.S., with three plates and upwards of 350 illustrations. Imperial 8vo, pp. xvi—382. (London: Crosby, Lockwood, and Son. 1893.) Price 18/-.

The introductory chapter deals with Elementary Optics. A short extract from the section, which treats of REFLECTION, will show the thoroughly practical manner in which the subject is treated:—"Let us examine the course taken by rays falling on a plane mirror. Let AB , Fig. 25, be the mirror, C a luminous point; a ray emanating from the point, and striking the mirror at D , will make, with the perpendicular to the surface of the mirror at that point (which perpendicular is termed the *normal*), an angle EDF , called the angle of reflection, equal to CDE , the angle of incidence. Other rays, CG , GH , emanating from the same point, make again, with the normals GI , HJ , the angles of reflection IGK , JHL equal to the angles of incidence CGI and CHJ ; thus all the reflected rays seem to diverge from a point C' , which has no real existence, and is called the virtual image of C . Its position, as compared with that of C , is symmetrical with regard to the mirror."

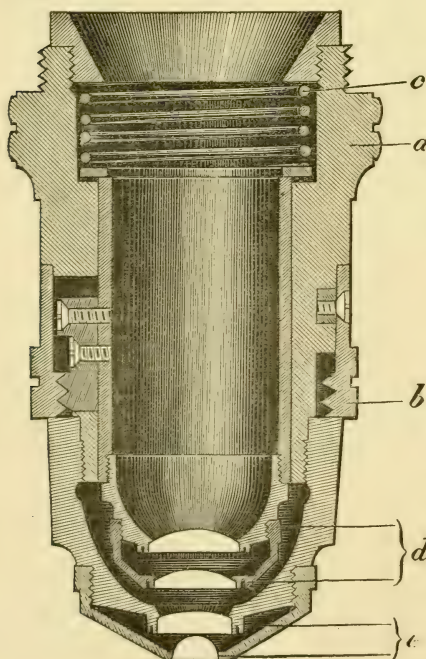


Fig. 26.

This section is followed by others on Refraction, Lenses and their properties, Spherical aberration, etc., and explained by a number of diagrams. Chapters II. and III. describe Prof. Abbe's Theory of Microscopic Vision, and experiments on its application. Book I. treats of the microscope, its optical parts, stage, illumination, accessory apparatus, etc., etc. Here we have a good sectional view of an objective with correction collar. "In 1829, Amici first noticed that high-power objectives, which give a perfectly clear image

when objects, not covered with a glass, were examined, but did not give so good a one when the object was covered, and that the clearness of the image increased and diminished according to the thickness of the cover-glass. To remedy this defect, which resulted from spherical aberration, Amici constructed his objectives in such a manner that they could all be used with cover-glasses of a definite thickness.

"In 1837, the celebrated English optician Ross, though ignorant of the discovery of Amici, made the same observation, and, to remedy the defect, he invented correction objectives. In this kind of objective, the two upper lenses (Fig. 26, *d.*) occupy an invariable position with regard to one another. They are fixed in a movable tube, and can be made to recede from, or approach, the lower part, which is fixed, and carries the single or double frontal *e*. By turning the ring *a*, the upper lenses rise or fall, and the coiled, spiral spring *c* regulates the small inequalities of the screw, and, above all, prevents the 'back lash,' which is always produced when the sense of motion is changed."

Fig. 27 shows how a microscope, which can be inclined, is

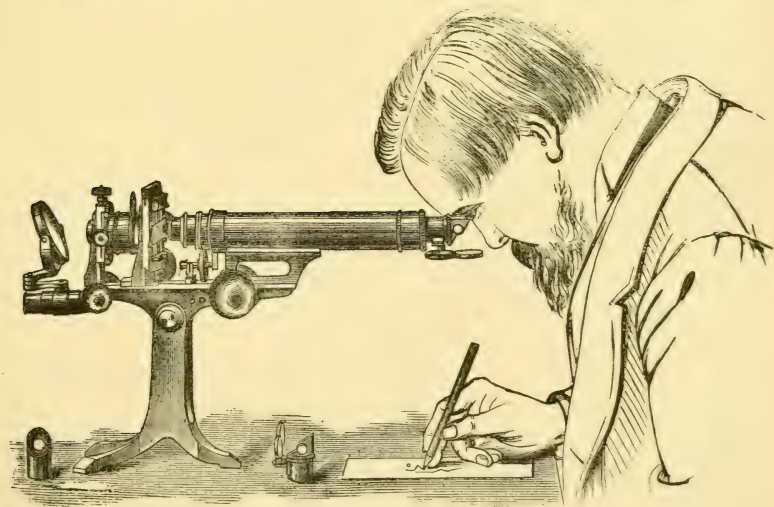


Fig. 27.

arranged for adapting the camera lucida. When this apparatus is

used, the light of the microscope must be so regulated that it is of an intensity as nearly as possible equal to that from the paper on which the drawing is being made ; when the light from the paper is more intense than that of the microscope, it is impossible to distinguish the point of the pencil sufficiently well. In this case the light from the paper must be reduced by means of a screw.

Passing Book II., which treats briefly of simple microscopes, and of projection—Solar, Gas, or Photo-electric—microscopes, we come to Book III., which describes to the reader what should be the situation and arrangement of the work-room ; choice of light ; hygienic rules for microscopical research, in which we are told there is absolutely no foundation for the statement that microscopical research is injurious to the eyes, and that no trouble need be feared by anyone who will take heed of the following advice :

“ 1.—Do not make observations directly after a meal.

2.—Let the field of the microscope be comfortably illuminated. Always avoid brilliant illumination, and on no account use [direct] solar light for ordinary observation. It is only during experiments with polarised light, photo-micrography, and with monochromatic light, that solar light can really be employed to advantage.

3.—As soon as your eyes feel at all fatigued, suspend your observations at once. This is of the greatest importance.

4.—An excellent hygienic rule, which has greatly assisted us during the last six years, is to wash the eyes thoroughly every morning with warm water. We use a litre ($1\frac{3}{4}$ pints) of water for this daily ablution. The warm water thus employed produces at first a very slight congestion, followed immediately by an excellent reaction. We cannot too strongly recommend this washing, which rests the eyes. Cold water, on the other hand, gives a momentary calm, followed afterwards by a congestion of the visual organ.”

Descriptions, covering nearly 100 pages, are given of instruments manufactured by various American, Continental, and English opticians ; this portion of the work is very fully illustrated. Concise instructions are given on Photo-Micrography, in relation to which a variety of apparatus is illustrated ; these are mostly of continental manufacture. The causes of error in microscopical observations are pointed out, one of them being *Musæ volitantes*, popularly

known as Flying Flies, represented in Fig. 28. These “are more visible and tenacious one day than another; a brilliant light or physical fatigue renders them more apparent. We sometimes perceive these flies with ordinary light, when looking up at the sky, or at a strongly illuminated surface, such as the ground covered with snow. At other times, notwithstanding the most fatiguing examination of diatoms, we have remained for entire weeks without being aware of their existence.”

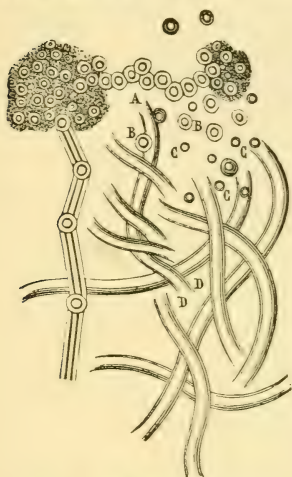


Fig. 28.—*Musca volitantes*.

In Book IV. we have: Chapter 1, General Rules for preparing micro-objects, with descriptions of the various Aqueous and Oily, Resinous and Chemical, media; Chemical and

Staining Reagents, and instruments used in the preparation of objects, etc., etc. Chapter 2 is entitled the Microscopist's Library, in which the author has “collected together a number of books which the microscopist, and especially the botanical microscopist, may, with advantage, consult,” but judging from the very meagre list, Foreign and English (about three dozen works are named), we cannot think his library is overdone with periodical literature, as neither the *Journal of the Quekett Club*, nor our own *Journal*, are mentioned, nor are the *American Monthly Microscopical Journal* or *The Microscope*.

Book V. treats of the microscope in the past and in the future. Here, we are told, “an early form of microscope consisted of a magnifying glass set in a mount, and carried on a small foot. A needle was fixed at a short distance from the lens, and the object for examination was stuck on the point of a needle. It was this sort of microscope which gave rise to the microscope of Leeuwenhoek, and with which this illustrious microscopist made his splendid discoveries.

Leeuwenhoek's microscope, Figs. 29 and 30, differed from that above described in the perfection of his lenses, which magnified

highly, and in the fact that the point which carried the object was

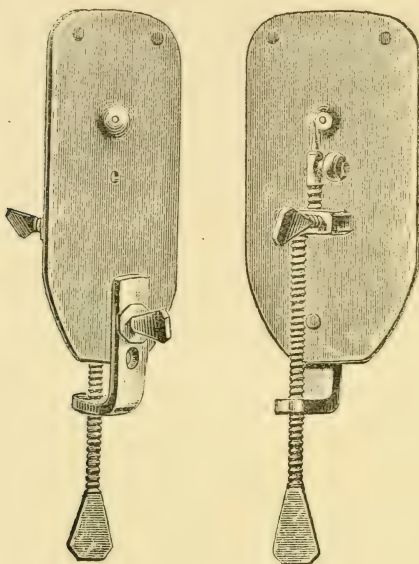


Fig. 29.

Fig. 30.

Leeuwenhoek's Microscope.

capable of being raised or lowered by means of a screw or stem. The stem could spring back easily, and, by means of a small screw-nut, the object could be brought nearer to the lens, so as to be perfectly focussed. Leeuwenhoek had microscopes of differing magnifying power; one is known to have given 270 diameters. The lenses are bi-convex, and very well made.

The simple microscope was considerably improved by Wilson about 1740. His instrument was furnished with a mirror, and mounted on a stand as shown in Fig. 31. The object was placed between two glass slips, which were held tight between two small brass plates. A tube with a screw thread enabled the object to be raised or lowered so that it could be examined in distinct vision. A spiral spring, pressing from above, holds the plates close, and counteracts, at the same time, the back-lash of the screw used in focussing. His instrument was in great request, and imitated on all sides.

The invention of the Compound Microscope is attributed to Zaccharias Janssen, whose portrait we are enabled, through the

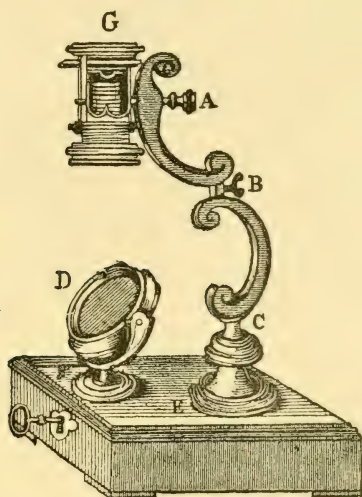


Fig. 31.—Wilson's Microscope, 1740.

courtesy of Mr. Wynne E. Baxter, to present to our readers. Janssen was a small optician of Middleburg, whose residence was attached to the Church.

An instrument called "Janssen's Microscope" is still preserved at Middleburg, which, according to Harting, can be referred back as far as his period without its being the original instrument. This instrument was shown at the Antwerp Exhibition.

The Microscope, Fig. 32, consists of four tubes, made of iron and soldered together, coated inside with tin. The outer tube *A* is of the greatest diameter, and contains the tubes *B* and *C*, which slide into it. The tube *B* contains, within itself, a fourth tube *B'*, having, at its lower end, a bi-convex objective lens of $3\frac{1}{2}$ inches focus. The ocular lens, of about 3 inches focus, is plano-convex, and is held in a wooden cell by a ring made of iron wire. The tube *C* which contains it is terminated at the upper end by a concave diaphragm. The tube *B'*, which contains the objective, is terminated at the upper extremity by a diaphragm, flush with the

end. The tube *B* is terminated at its lower extremity by a diaphragm. In using this instrument *B'* is pushed right down into *B*, and the tubes, *B* and *C* are drawn out as far as possible from the exterior tube *A*, and the instrument is directed towards the

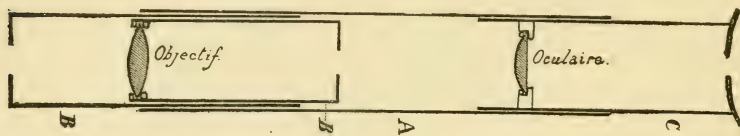


Fig. 32.—Janssen's Microscope.

object. In the foregoing notes we believe we have taken a fair survey of the book, and in doing so have selected such of the illustrations, kindly lent to us by Mr. Baxter, as we thought would be most interesting to our readers. The article on the Future of the Microscope is comprised in a long letter by Dr. S. Czapski, and will be found towards the end of the volume; this we will leave our readers to peruse at leisure from the volume itself. There is a full and comprehensive Glossary-Index.

Staining Insect Tissues.

AT a meeting of the Entomological Club of the American Association for the Advancement of Science, 1891, Mr. Smith made a few remarks on Staining Insect Tissues. He had found considerable trouble in his studies on differentiating parts, and especially those structures that tend to become transparent. After considerable experimentation he had found *nigrosin* one of the most satisfactory stains for trachea and glands, and many of the membranaceous structures. It does not touch chitine. By the use of this stain he had followed the trachea to the tips of the antennæ and into the labella of flies. Saffronin is another valuable stain, and especially for chitinous structures, for which it seemed to have a special affinity. Combining nigrosin and saffronin often gives very pretty results. Care should be exercised not to leave the objects in saffronin too long, as it is apt to result in a uniform and too intense colour, which is hard to get rid of. Hæmatoxylin gave very poor results, and he does not look on it with favour. Eosin is excellent where only a slight stain is desired, and has given some beautiful results. The use of such methods in studies admitting of them will solve many problems that are still obscure.—*Canadian Entomologist*.

Injecting Small Animals for Microscopical Purposes.

BY R. N. REYNOLDS, M.D., DETROIT, MICH.

HAVING experienced much disappointment in the results of injections with injecting gelatin purchased, I experimented until I found proportions of the materials which would give uniform, beautiful specimens, and at less than half the cost of the old method. For thirteen ounces of gelatin costing about fifty cents., which would be sufficient for a full-grown cat, take of gelatin (such as is sold in checkered packages in grocery stores) 600 grains, place in a pint fruit-jar and add to it five ounces of cold water ; cover, and set aside for an hour or over-night. Put into another air-tight jar, (No. 40) carmine, 400 grains ; water, 4 oz. ; and stronger water of ammonia (*ammonia fort.*), 4 drams. Cover tightly and set aside.

When ready to proceed, place the jar of gelatin into a water-bath and heat until the gelatin is melted ; then strain through a fine cloth into a clean jar (a linen handkerchief makes a good strainer). Next insert a wad of absorbent cotton into the neck of the funnel, and through it filter the carmine solution into a jar of gelatin which has been kept warm in the water-bath. If the cotton plug proves too tight to allow all the carmine to pass through, decant the remainder into the jar it came from, replacing the cotton plug by a looser one.

After all the carmine has passed through into the gelatin, clean the funnel, and place in it a fresh plug of cotton, pour into the funnel some water, and with a rod press down the cotton until the water goes through drop by drop. *This test water is not to be allowed to drop into the gelatin.* Then replace the funnel so that it hangs over the gelatin, putting into the funnel the following to granulate the carmine :—Water, 2 oz. ; glacial acetic acid, 4 drs. ; and while this is dropping in, the gelatin must be continuously stirred. By the time the whole of the acid has passed in, the gelatin will be changed from a dark lilac to a bright scarlet colour, and is then ready for use.

The exact quantity of acid that it will take to bring about this

change is not constant. It depends on the strength of the ammonia used, the amount of ammonia which has escaped during the manipulations, and also on the strength of the acid.

The change in colour may be plainly seen, as it is quite marked and sudden, by looking, not into the mouth of the jar, but on its side, where the light is strongest, and when the change in colour comes the gelatin is ready for use ; but it will do no harm to use all the acid prepared. A greater quantity of water with weaker ammonia would do, but it is best to use a standard article. So also with the acid. The ordinary acetic acid would do if we used more of it and waited for the change in colour. If we put in too little acid, the fluid would be too dark, and the colour would pass through the walls of the blood-vessels, staining the intervening tissues.

For injecting we need the following :—

- 1.—Injecting-syringe, with canula and stopcock.
- 2.—Curved needle, threaded with No. 10 Chinese silk.
- 3.—Some ordinary strong parcel twine.
- 4.—A sharp-pointed knife.
- 5.—An ordinary wash-basin.
- 6.—A kettle of hot water.
- 7.—A pail of ice water.
- 8.—A starch or other box, with sliding lid.
- 9.—Some chloroform.

We should use much more gelatin solution than would fill the animal's blood-vessels, because a considerable quantity should be allowed to pass through and out of the animal to wash out the remains of the blood, which if left in would turn black ; and also because a greater amount should be forced and left in, to ensure well-filled capillaries in our sections.

When ready to inject, the jar of gelatin, the syringe, and parts should be kept in warm water.

The animal should be placed in the box and the lid closed. Next pour some chloroform on a cloth and drop it into the box, and wait for the animal to cease moving about. After narcosis is complete, and before the animal ceases to breathe, it should be removed from the box. The operator should with the thumb and finger seize the skin of the belly to lift the parts from the intes-

tines while the knife is passed through, opening from near the hind legs, well up between the fore-legs. Cut the diaphragm, to allow the fore-legs to spread. Seize the heart with one hand, while its apex is cut off with the knife in the other hand.

Hold the animal up alternately by head and tail to allow blood to drain out ; wash away the blood. Pass the knife up between the heart and pericardium, slitting up the latter so that it may be pushed up to expose the aorta. Place the animal in a basin of warm water. Do not hurry ; there will be plenty of time for every move.

If we have cut only a quarter or three-eighths of an inch from the heart, we have opened the left ventricle only, and cannot then pass the syringe canula into the right ventricle in error.

Hold the heart in the left hand, while with the right the detached syringe canula is run up through the heart, its point passing out into the aorta, using care not to pass it too far up, else it might puncture the arch of the aorta. Now with the right hand pass the curved needle under the aorta, through the tissues between it and the superior vena cava.

The canula may now be allowed to drop out of the heart while the thread is drawn partly through, and the first half of the surgeon's knot is loosely tied ; then replace the canula and tighten the thread on it, completing the knot. Bring the long ends of the thread up over the hook on the canula, tighten, and again tie it.

The nose of the stop-cock is now twisted tightly into the canula, while the canula is held in one hand to prevent twisting the aorta. Drop the stop-cock so that it is covered with the warm water while the syringe is being filled.

When the syringe is slowly filled, close its nose with a finger, rinse the gelatin from the outside of the syringe ; then, placing its point under water, enter it into the stop-cock, holding the latter firmly while the syringe is twisted tightly into it. Hold the nose of the syringe tightly in the one hand, while the piston is managed with the other. This is to prevent damage to the aorta.

Force the solution *very slowly* into the animal. We will notice a rapid change in the colour of the animal's nose, pads of the feet, intestines, etc. ; and very often the animal will kick and twitch about for five or six minutes, although it was dead some time before the injecting began.

Do not quite empty the syringe each time, as some air would be forced out with the last of the fluid. Close the stop-cock, refill the syringe and proceed as before, bearing in mind that injecting too rapidly is apt to burst some vessel and spoil the subject. When you think that about sufficient has been forced in, pass a string around the heart and tie it tightly around the canula to prevent further escape ; then force in from one-half to one ounce more, depending on the size of the animal. This is to prevent the capillaries being emptied by the rigor-mortis, which will set in later. (The syringe should be *slowly* filled, else air will be drawn in at the back and be forced into the animal.) Now close the stop-cock, remove the syringe, and hold the animal in a stream of water from the tap to wash away stains from the outside and the abdominal cavity. It should next be placed for some hours or over-night in ice-water. If desired to harden, ice-cold Müller's fluid is to be preferred. After which, it may be dissected and the parts desired may be placed in ordinary alcohol. After a few hours this alcohol may be turned off, to be replaced by fresh alcohol. In this way the brain and kidneys will be ready for cutting in forty-eight hours.

It is a mistake to expect tissues to harden in the second or third alcohol. It is not length of time that is necessary to harden, but the several changes of alcohol to get rid of water from the tissues.

After hardening, we may cut our sections, place them for a few moments in oil of cloves ; then transfer them one at a time to a clean pad of tissue paper ; put a drop or two of benzole on to a slide ; lay the section into the benzole (which will drive out the air-bubbles) ; apply a drop of Canada balsam, then the cover-glass, and our specimen is ready for the microscope.

If our balsam has been properly prepared, and the slide is deposited in a warm place, it will soon harden into a permanent mount, to be admired by lovers of the beautiful.

We cannot well secure the brain and spinal cord by sawing. I find the following to work nicely :—Cut the neck off close to the skull, remove the skin and muscle from the top of the head ; then with a screw-driver or similar tool commence at the foramen magnum ; pick away the skull piece by piece ; this is done by

inserting the tool between the bone and the covering of the brain, (the Dura Mater) ; then pulling away the piece of bone, we run no risk of damaging the brain.

After the bone has been removed from the top, split up the Dura Mater, and with a pair of forceps pull out the bony septum from between the cerebrum and cerebellum ; next, a little shaking will show the nerve attachments, which hold the brain on its under side. After these are cut the brain will fall out, and may be placed in ordinary alcohol to harden.

The spinal cord may be easily secured by using a wide chisel. First place the trunk of the animal on its back, and with the chisel cut the ribs from both sides of the vertebræ, letting the chisel pass close to the vertebræ, and down through to the work-bench. Next, let the slice containing the vertebræ lay on its side, set the chisel on to the bone over the spinal cord, and with the hammer tap on to the handle of the chisel, to make a longitudinal fracture along the cord. Continue this the whole length of the cord.

Turn over the slice and repeat the fracture along the opposite side ; then seize the two sides of the bone, one in each hand, and pull them apart. The cord will then be set free with the nerve-roots hanging to it.

From various experiments respecting a connection between thunderstorms and the souring of milk, Prof. H. W. Conn draws the conclusion that electricity is not of itself capable of souring milk, or even of materially hastening the process ; nor can the ozone developed during the thunderstorm be looked upon as of any great importance. It seems probable that the connection between the thunderstorm and the souring of milk is one of a different character. Bacteria grow most rapidly in the warm, sultry conditions which usually precede a thunderstorm, and it will frequently happen that the thunderstorm and the souring occur together, not because the thunder has hastened the souring, but rather because the climatic conditions, which have brought the storm, have, at the same time, been such as to cause unusually rapid bacteria growth.

Seedlings.*

By the Right Hon. Sir JOHN LUBBOCK, Bart., M.P., F.R.S.,
D.C.L., LL.D., etc.

IT is with much pleasure that we give our readers as full a notice of these two most interesting volumes as the limited space at our disposal will allow. We are sure that all botanists will agree with the author when he tells us in the opening paragraph of his preface that "the germination of plants is not the least interesting portion of their life-history, but it has not yet attracted the attention it deserves. The forms of cotyledons, and the fact that they differ so much from the subsequent leaves, had, of course, been alluded to more fully in botanical works, but no explanation had been offered, and Klebs, in a recent memoir, expressly states that the problem is still an enigma."

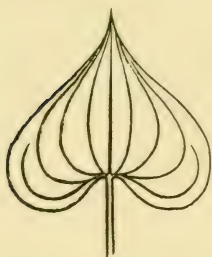


Fig. 33.—Leaf of *Tamus*, to show the curved course of the veins.



Fig. 34.—Leaf of Sycamore, to show the straight course of the veins.

Speaking of the FORMS OF LEAVES, and of their endless differences, the author tells us "vertical leaves, for instance, are generally long and narrow, horizontal ones have a tendency towards width, which brings the centre of gravity nearer to the points of support. Wide leaves, again, are sometimes heart-shaped, sometimes palmate. The former shape is obviously that

* "A Contribution to our Knowledge of Seedlings," by the Right Hon. Sir John Lubbock, Bart., M.P., F.R.S., D.C.L., LL.D., with 684 figures in the text. In two vols., 8vo, pp. viii.—608 + 646. (London: Kegan Paul, Trench, Trübner and Co., Ltd., Paternoster House, Charing Cross Road. 1892.) Price, 36/- nett.

which would arise if a linear leaf were gradually widened at the base ; and I have pointed out that in many species with palmate leaves—for instance, species of *Passiflora*, *Cephalandra*, *Hibiscus*, etc.—the first, or few first leaves, are entire, and more or less cordate. The cordate form, then, appears to be the early, the palmate the later form. But how has the palmate form arisen ?

“The origin is, perhaps, connected with the manner in which the leaves are folded up, more or less like a fan, in the bud, so as to save space. Another advantage, perhaps, is that the cordate leaves with veins following the curvature of the leaf, as, for instance, in *Tamus*, Fig. 33, the vascular bundles pursue necessarily a curved course ; while in palmate leaves, as in *Acer*, Fig. 34, the veins are straight, and it is clearly an advantage that the main channels which convey the nutritive fluid should hold a direct course.”



Fig. 35.—Seedling of *Foeniculum vulgare*, half nat. size.

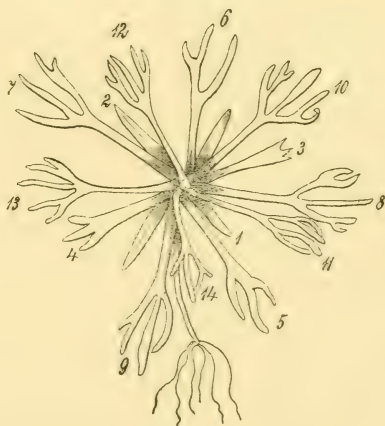


Fig. 36.—Seedling of *Ceratocephalus fulcatus*, nat. size. The numerals indicate the successive leaves.

We now turn to the FORMS OF COTYLEDONS, and are told that

“no one who has ever looked at a young plant can have failed to be struck by the contrast they afford to the older specimens belonging to the same species. This arises partly from the contrast which the cotyledons, or end leaves, afford, not only to the final leaves, but even to those by which they are immediately followed.”



Fig. 37.—Seedling of *Impatiens balsamina*, half nat. size.



Fig. 38.—Seedling of *Menispermum canadense*, half nat. size.

Some cotyledons are narrow, as in *Fœniculum*, Fig. 35, and *Ceratocephalus*, Fig. 36. In *Ceratocephalus* (and in many other species) the order of growth of each successive leaf is shown, and its shape described. Thus, leaves

- 1, 2 are linear, entire, similar to the cotyledons.
- 3 Spathulate ; apex tridentate.
- 4 Spathulate, trifid.
- 5, 6 Cuneate, unequally trilobed.
- 7, 8 Cuneate, tripartite ; segments linear, lateral ones bifid at apex.
- 9 Tripartite, with linear segments.
- 10 Tripartite, lateral segments unequally lobed.
- 11 Doubly bipartite.

12 Tripartite ; lateral segments bifid.

13 Similar.

14 Cuneate, tripartite, lateral segments unequal.

Some cotyledons are broad, as in *Impatiens*, Fig. 37 ; in other species we find narrow cotyledons and broad leaves, as in *Menispermum*, Fig 38, while in others the cotyledons are broad and the leaves narrow, as in *Hakea*, Fig. 39.



Fig. 39.—Seedling of *Hakea acicularis*, half nat. size.

In some cases instances of broad and narrow cotyledons may be found in the same family, as in Chickweed, and Pink, and sometimes even in the same genus, as *Galium Saccharatum* and *Gallium Aparine*. In some cases, again, the two cotyledons are unequal, as in Mustard, Cabbage, etc. Sometimes the two halves of each cotyledon are unequal, as in *Geranium*, and indeed their variety appears to be very considerable.

Turning now to the embryo, some exceedingly interesting descriptions of its growth are given, as, for example, in *Acer*, Fig. 40, the embryo originates in a short tubular cavity opposite the micropyle, and is at first straight, with an extremely short tubinate radicle, and ovate, obtuse, closely adpressed cotyledons.

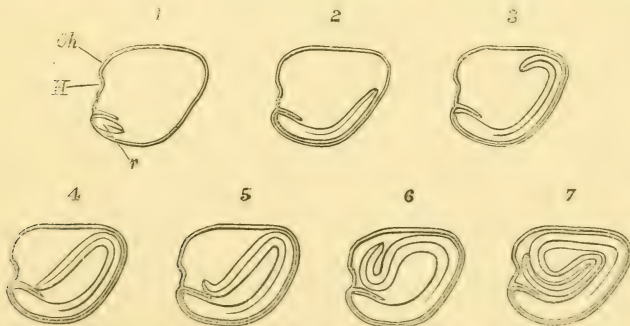


Fig. 40.—*Acer Pseudo-Platanus*. Sections of seed in seven successive stages, showing growth of embryo, $\times 3$.

As growth continues the embryo extends itself along the lower

side of the seed, and curves with it, becoming gradually lanceolate, or oblong lanceolate (Fig 40, 2). When the cotyledons have reached the upper, narrow end of the seed, the curvature of the wall turns them down again on themselves (Fig. 40, 3). This growth is continued until the tips reach the radicle again, and the ultimate arrangement of the embryo differs according to whether they then curve inwards or outwards. This again seems to depend on the exact direction of the growth of the cotyledons; if they strike (Fig. 40, 5) against the process which encloses the radicle, then their general direction naturally carries them outwards, until the wall of the seed again turns them upwards, so that they become plicate; if, on the contrary, the tips of the cotyledons press just within the micropylar process and touch the radicle, then they are compelled to grow in the opposite direction, and they become spirally coiled. In the specimens examined the latter arrangement was exceptional.



Fig. 41.—Seedling of *Lasiopetalum ferrugineum*, half nat. size.

With respect to the growth of FIRST LEAVES we are told:—
“In species with trifoliate leaves, the first leaf is generally simple. When mature leaves are pinnate, the first ones are generally trifoliate; and when the final leaves are bipinnate, the first ones are generally pinnate. In most cases the first leaves are simpler than those which follow.” In species however, from very dry localities, the reverse is often the case, as in *Lasiopetalum*, Fig. 41.

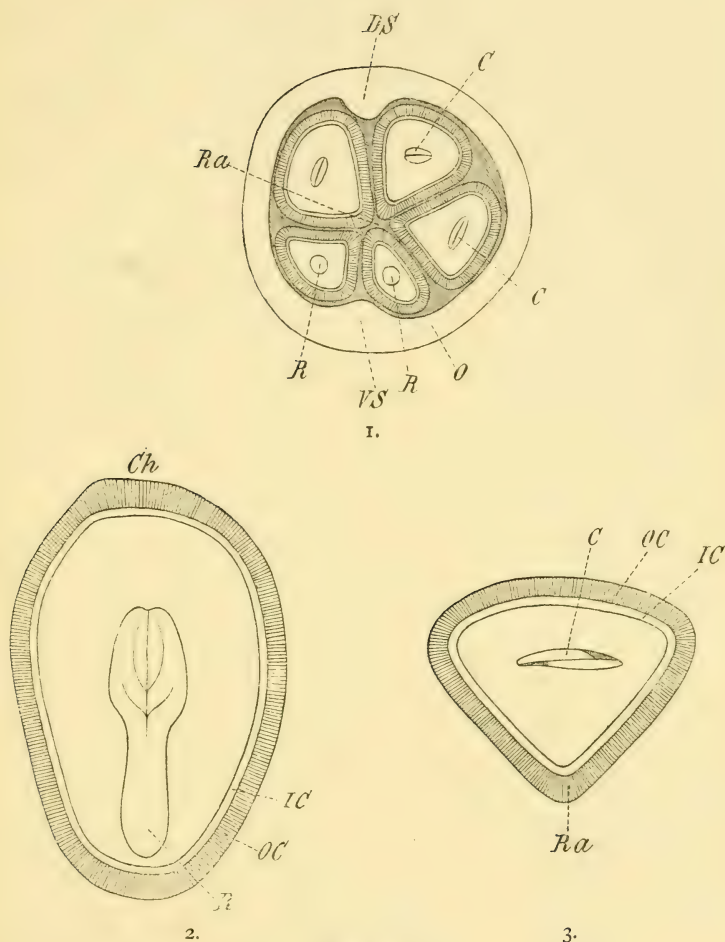


Fig. 42.

Berberis Aquifolium.

- 1.—Transverse section of fruit, $\times 5$. D.S., dorsal suture; Ra., raphe; R.R., radicles; C.C., cotyledons; V.S., ventral suture; O., ovary wall or pericarp.
- 2.—Longitudinal section of seed, $\times 10$. Ch., chalaza; R., radicle; O.C., outer coat (testa); I.C., inner coat (tegmen).
- 3.—Transverse section of seed, $\times 10$. C., cotyledon; O.C., testa; I.C., tegmen; Ra., raphe.

An immense number of seedlings are described and figured in

these volumes, some few of which figures, by the courtesy of the publishers, Messrs. Kegan Paul, Trench, Trübner and Co., we are enabled to present to our readers. To give an idea of the thoroughness with which the author has performed his task, we turn to the BERBERIDEÆ, and briefly abstract the general description:—"The fruit of the Berberideæ is a berry or capsule. The ovules are two, indefinite, very rarely solitary. . . The seeds contain a copious, fleshy, or somewhat hard endosperm; and the embryo is frequently small. . . The cotyledons are



Fig. 43.—*Berberis Aquifolium*, natural size.

generally oblong, obtuse, shortly petiolate or sessile, deep green, glabrous, sub-coreaceous, with a few slender, ascending, and generally slightly emarginate veins."

³ The illustration, Fig. 42, shows sections of fruit and seed of *Berberis aquifolium*.

Fig. 43 shows a young plant of the same drawn natural size. The cotyledons are described as oblong, obtusely pointed, narrowed into the petiole, glabrous, 1.5 cm. long, 5 mm. broad. Leaves simple in the seedling stage.

- 1.—Reniform, cuspidate, minutely spinous-serrulate.
- 2.—Reniform, cuspidate, finely spinous-serrate, fine nerved at the base.
- 3-4.—Cordate, finely spinous-serrate, fine nerved at the base.
- 5.—In a second specimen, unequally bifoliate.
- 6.—Trifoliate. Subsequent forms three to five foliate, and ultimate leaves 3 to 9 foliate.

Turning now to the CRUCIFERÆ, we select as an example Fig. 44, which shows the fruit and seed of *Ochthodium ægyptiacum*.

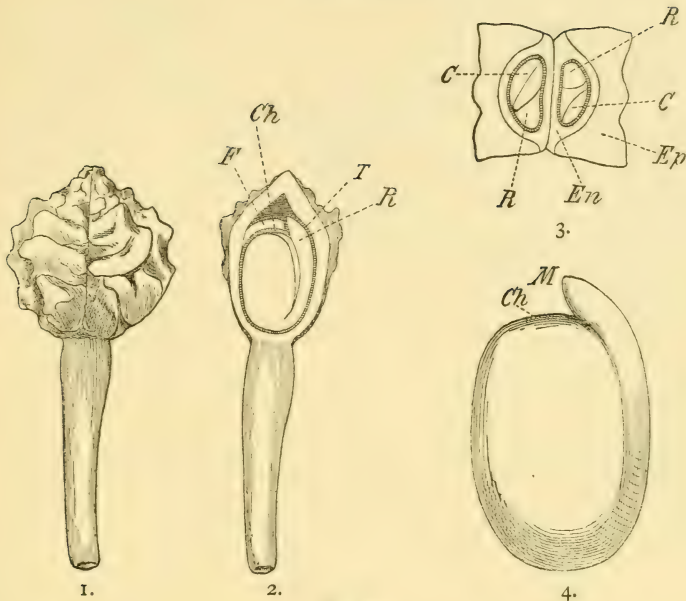


Fig. 44.

Ochthodium aegyptiacum.

- 1.—Fruit on its pedicel, $\times 6$, lateral aspect.
- 2.—Longitudinal section of fruit, $\times 6$. *F.*, funiculus; *T.*, testa; *R.*, radicle; *Ch.*, chalaza.
- 3.—Transverse section of fruit, $\times 6$. *C.C.*, cotyledons; *R.R.*, radicles; *Ep.*, epicarp; *En.*, endocarp.
- 4.—Seed, $\times 15$. *Ch.*, chalaza; *M.*, micropyle.

One more example from this important work is all our space will allow. Fig. 45 shows the seed of *Bryonia laciniosa*, and Fig. 46 a young seedling of the same, half natural size.

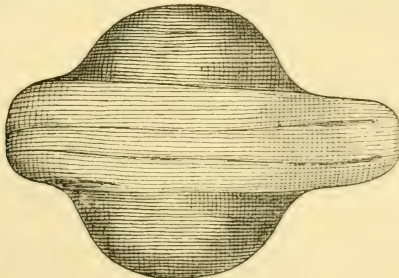


Fig. 45A.

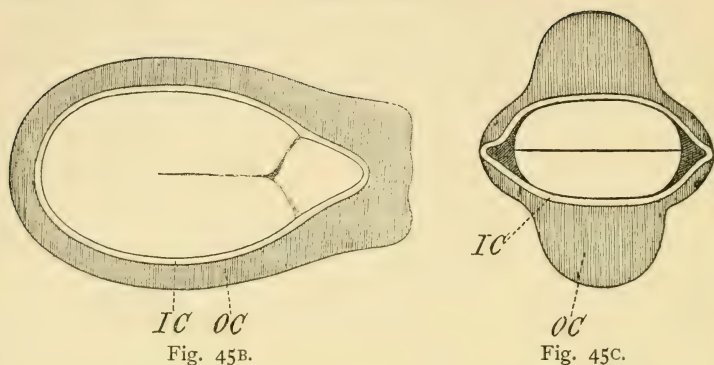


Fig. 45.—*Bryonia laciniosa*. A, seed, $\times 10$; B, longitudinal section of seed; O.C., testa; I.C., tegmen, $\times 10$; C, transverse section of seed, $\times 10$.

In making these selections we have confined our attention to Vol. I., although Vol. II. would have proved equally prolific of good and interesting examples. It will be noticed, too, that we have generally selected illustrations where sections of seeds are



Fig. 46.—*Bryonia laciniosa*, half nat. size.

also shown. This we have done in a great measure to induce our readers to adopt what we think will be, to a great number of them, an entirely new branch of study, viz., the microscopical structure of the seeds of plants.

The subject of seeds and seedlings is a very vast one, and we earnestly hope that Sir John Lubbock's marvellous work may be the means of suggesting an entirely new field of work to great numbers of microscopists. In looking at these books, with their nearly 700 illustrations, we cannot but admire the unwearied diligence and careful study displayed in their production.

A Midwinter Month by the Mediterranean. Last Week.

BY G. H. BRYAN, M.A., Cantab.

PART IV.—GRASSE AND HYÈRES.

At Grasse I stayed one night at the Grand Hotel, subsequently the residence of our Queen. Grasse is a picturesque old town, built high on the side of a long range of hills about 12 miles from the sea, and reached by a branch line of railway from Cannes. The chief manufactures of the place are perfumes and preserved fruits, so that, although the streets are many of them very narrow, they are fragrant with the scent of the numerous perfumeries. The road from the station ascends in a series of zigzags, crossing a new line of rail in process of construction, and east of the town is a fine boulevard leading past the Grand Hotel. From this point is a fine view over the Golfe de la Napoule and the Esterel Mountains. In the olive plantations the ground is everywhere carpeted with leaves of *Anemone coronaria*, and a white kind of wild radish (*Raphanistrum Landra*) grows by the roadsides. I visited the perfumery and confectionery works in Grasse, but there was little or nothing going on at them.

On the following morning I made my way up the hills at the back. After emerging from the olive plantations I came out on the open scrub and soon reached an aqueduct, quite recently con-

structed for the purpose of supplying Grasse with water. It runs along the side of the hill, dipping down and up in a syphon at a depression in the mountain side. Beside this I found *Polygala Nicæensis*, *Osyris alba*, and a fine pink Candytuft (*Iberis linifolia*), from which I have raised seedlings that now form splendid, compact masses of flower, fully a couple of feet in diameter. I should be pleased to send seeds to any readers who may care to send an envelope for them.

By following the watercourse, I emerged in a few minutes on a well made road leading along the hills, which were here covered with *Cistus albidus* (now in fruit), small oaks, and other shrubs. A little way along the road I found large shells of *Zonites cellarius* and a couple of big brown ants (*Formica cruentata*). In returning I followed the road down to Grasse past an ornamental fountain at the end of the aqueduct, and some way lower down the road found plants of *Salvia clandestina*.

From Grasse I took the train to Hyères, spending an hour enjoying the view from the Promenade de la Croisette at Cannes while waiting for the train. Cannes itself has grown so large and has been so built over of late, that I am told it is difficult now to get country walks in the neighbourhood. Many years ago it was possible to find anemones and pink maiden tulips (*Tulipa Clusiana*) growing wild at the Croisette close to Cannes.

The train did not reach Hyères till dusk, and on driving up to the hotel I was much charmed with the effect of the electric arc lamps which now illuminate the avenue of palm trees between the station and the town.

Hyères, the most southerly station on the Riviera, is a small town at the foot of a line of hills, of heights ranging up to 800 feet. The sea is about three miles away, but is seen from the town, with the islands of Hyères (the *Stæchades* of the ancient Romans) in the distance.

The following morning I walked up the Colline des Oiseaux. The way lay along the main road to Costebelle for some distance past the station, diverging by a rough cart-track nearly opposite the Hermitage. In a cottage garden near here some fine double narcissus were in flower which I remembered having seen there fourteen years previously. I had some little difficulty

in finding my way up the hill, for I lost the right path several times, and progress was then much impeded by the small prickly oaks (*Quercus coccifera*), interspersed here and there with the equally prickly butcher's broom (*Ruscus aculeatus*). I saw the leaves of *Ophrys fusca* here and there, and some more of the candytuft (*Iberis linifolia*) in flower. There were a good many shells of *Bulimus decollatus* (all broken off at the point), *Zonites cellarius*, and a kind of *Cyclostoma* about here, and in a few shady places the ground was white with hoar frost.

A little further up a large fritillary butterfly was seen flying about, but not caught. The top of the hill, about a thousand feet high, was soon reached, and from among the arbutus trees (*A. unedo*) there was an extensive view comprising the islands of Hyères and the Salines, or salt marshes, on one side, and on the other the range of hills behind Hyères known as "Les Maures," all standing out clear in the bright sunshine. From the top I came down in the direction of Carqueiranne, finding on the way some more shells of *Zonites*, *Coronilla juncea*, marigolds (*Calendula arvensis*), the white *Raphanistrum Landra*, *Alyssum maritimum*, etc. After passing a small farmhouse, and traversing a few olive plantations, I emerged on the road to Carqueiranne. This skirts along near the shore, but the views are much impaired by several villas on either side.

In a bank by the roadside I found fine plants of *Erodium romanum*, and in digging one up accidentally destroyed a small trap-door spider's nest of the "cork" type—i.e., with tight-fitting door. The return was through some olive plantations by Costebelle, where the Hotel d'Albion forms a hideous eyesore, here the ground was carpeted with large daisies (*Bellis sylvestris*); further on, a little before reaching the railway station, I noticed in a bank several trap-door spiders' nests with thin "wafer" doors, constructed by *Nemesia congener*, a species peculiar to the neighbourhood of Hyères.

In the afternoon I followed a path called the Chemin de St. Bernard, leading round the rock on whose slopes Hyères is built and which is crowned by the remains of an old castle. On joining the ridge behind the castle hill, I found a quantity of heads of the grass, *Lagurus ovatus*, in a dried state. In a wall near here

grew the Jersey fern (*Grammitis leotophylla*). Turning in the direction of the Pic du Fenouillet, I found large patches of the dwarf annual daisy (*Bellis annua*) as much smaller than our English *B. perennis* as the latter is smaller than *B. sylvestris*. Near here *Oxalis Libyca* grew in abundance, and also *Ranunculus chærophyllus*. In a bank a little further on were the tall dried remains of the flowering heads of *Asphodelus ramosus*. The path next skirted the side of a hill covered with cork trees (*Quercus suber*), and overlooking the valley of the river Gapeau. Here I was fortunate enough to come upon a specimen of that gorgeous beetle, *Calosoma sycophantha*, the larvæ of which live in the nests of the Procession Caterpillars (*Cnethocampa pityocampa* and *processionea*), and do a great deal of good by consuming large numbers of these destructive caterpillars. The beetle is truly magnificent, its body being about $1\frac{1}{4}$ inches in length, and its green elytra in a bright light reflecting tints of green, purple, orange, crimson, and nearly every colour of the rainbow. It is considered rather a rarity. There were also some fine ferns of the acute variety of *Asplenium adiantum-nigrum*. Returning down the castle hill, I found more large nests of the trap-door spider, *N. congener*. The sunset that evening was very brilliant.

The following morning I walked out through the Place de la Rade to the eastern end of the town. Here a good many villas have been built, but these have of late years been letting badly. Very soon I found a path turning up on to the hills behind, and I followed along the ridge covered with cork trees, arriving finally at the point where I had found the asphodels the day before. Here I came upon the small leaves of an *Orchis morio-laxiflora*. Climbing up a hill on the left, I saw a Red Admiral butterfly (*V. atalanta*) flying about, and on descending the shaly slopes the other side, where the heat of the sun was most intense, there were numerous grasshoppers of different sizes and colours jumping and flying about. One, *Ædipoda cærulescens*, with its deep-blue wings tipped with black; another species with delicate wings tinged with pale blue; and a third, *Acridium tartaricum*, with a wing expanse of about $2\frac{1}{2}$ inches, the wings tinged with lemon yellow, especially at their base. There were also a good many dried "earth-stars" (a fungus of the genus *Geaster*) about loose on the hillside. I

returned along the back of the castle hill down the winding alleys of the old town of Hyères.

In the afternoon of the same day (January 13) I left Hyères, gathering a few flowers of the little wild marigolds while waiting for the train as a last piece of collecting. The following morning, on the homeward journey, day broke with a leaden, sunless sky, showing that I was no longer in the "Sunny South," and that my "Midwinter Month" had come to an end.

The Parasitism of Protozoa in Carcinoma.*

BY JAMES GALLOWAY, A.M., M.D. ABER.,
F.R.C.S. Eng., M.R.C.P. Lond.

PROTOZOA AS PARASITES IN ANIMALS: TYPE, THE COCCIDIUM
OVIFORME IN RABBITS.

AS is now well known, the organisms which have been so frequently mentioned of late in connection with carcinoma belong to the division of the protozoa; and if I devote part of the time at my disposal to the study of a disease characterised during part of its course by the formation of tumours occurring in one of the lower animals, and undoubtedly caused by a certain protozoon, I shall carry out one of the suggestions of Sir James Paget, who pointed out that much light may be thrown on the formation of tumours in man by the study of growths in other organisms, and at the same time fulfil the purpose in my mind, when I commenced this inquiry, of obtaining a clear idea of some of the characteristics of the class of micro-organism which is now suggested as the cause of cancer.

The animal which I have chosen as the type of those infested by protozoa is the rabbit, principally because the life-history of the parasite, which is the cause of the disease, is very completely known, and may be looked upon as typical of many others allied to it in organisation. This affection of rabbits is brought about

* Abstracted from *The British Medical Journal*, by permission of the editor, to whom also we are indebted for the loan of the illustrations.

by the influence of one of the sporozoa (*Coccidium oviforme*, Leuckhart) which infests the intestine, bile ducts, and livers of diseased animals, in myriads. The disease is so common in certain hutches and warrens near London,* that the keepers recognise it readily, and distinguish it by the "wet snout," which the affected animals exhibit. It is most fatal in young rabbits; which become affected as soon as they cease to suckle and begin to eat green food. They lose flesh rapidly, suffer from enteritis of more or less acute character, and many die in from eight to fifteen days after the initial symptoms. The adult animal is more rarely infected, and, as a rule, resists the disease. The development of the parasite which brings about this very fatal disease may be considered in two stages, external to and within the host.

DEVELOPMENT EXTERNAL TO THE BODY.

The organism as it escapes from the alimentary canal consists of a firm, translucent cyst, enclosing a quantity of very granular protoplasm, which fills the whole cavity. The cyst, which is the striking feature of this period of development, is oval in shape, and measures about 36μ in length, and about 22μ in breadth. Very soon after expulsion, and often while within the

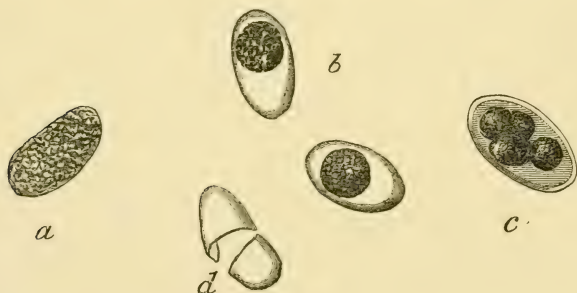


Fig. 47.—*a*, *Coccidium* showing capsule full of granular protoplasm; *b*, shows condensation of the protoplasm into one sphere, after two days' growth external to body; *c*, division of the single sphere into four daughter spherules, after four days' development; *d*, an empty ruptured cyst. (From Photographs magnified about 500.)

* Hutchinson, J., sen., *Archives of Surgery*, Vol. iii., 1891.

host, the protoplasmic contents contract, and form a sphere lying free within the cyst wall. Under suitable circumstances this ball of protoplasm pushes out projections, at first flattened, but soon becoming more distinct, till at length it divides into four distinct smaller spherules. Each of these protoplasmic masses becomes somewhat elongated, and forms, within itself, two crescentic germs lying in its long axis, leaving unutilised a small nuclear mass. A wall of less density than the outer cyst is formed round each of these groups of two germs, and then this stage of development is complete.

In this condition the parasite seems very resistant to injurious influences, and is capable of remaining alive for at least 6 months.

METHODS.

For the purpose of noting the changes which I have briefly mentioned, the parasites obtained from recently killed animals may be placed on a cover glass, and examined as a drop cultivation. The method which I have found most useful is that recommended by Professor Delépine, under the name of inter-lamellar films.* In this way the cycle of development, which I have described, will be accomplished in less than a week, the transformation into four daughter cells being noticeable on the third day, at the ordinary temperature of a room.

On commencing these observations, the ordinary bacteriological processes were made use of, with very little result. Tubes of sterilised blood serum, bouillon, and other media, were utilised, and the usual precautions observed. Subsequently the inoculated materials were placed in incubators at temperatures of 20°C. and 38°C. The more elaborate the precautions, however, the less result was obtained. The organism flourished best when freely exposed to the atmosphere. And as R. Pfeiffer observes, the presence of carbonic acid, and the change from the temperature of the body to the cooler external temperature, aid in their development. A warm temperature, and want of free aëration, modify and even prevent the changes described, and influences of this sort no doubt account for the very great discrepancies, as to time,

* See *Journ. Micros.*, Third Series, Vol. i., p. 339, 1891.

which may be noted between the accounts given by different observers. I have drawn attention to these facts to show how readily this important external cycle of development may be influenced.

DEVELOPMENT WITHIN THE BODY.

When an uninfected young rabbit swallows the parasite in the larval or resting condition just described with its food, the resisting capsules are acted on by the digestive ferments, so that the crescentic germs already mentioned become free. A new cycle of intense activity is now observed. These crescents become rounded, and probably acquire the power of locomotion. Most of the naked amoeboid forms of the organism divide into numerous small crescentic sporules, which, in their turn, also become free, and it seems probable that this "endogenous sporulation" may

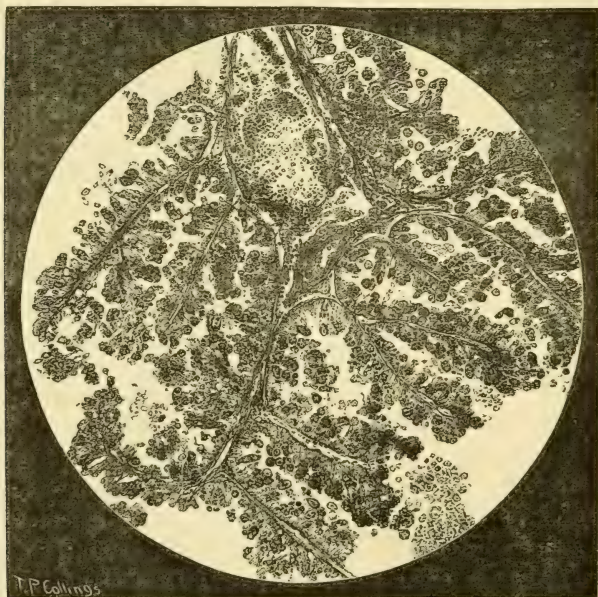


Fig. 48.—Adenoma from Rabbit's liver, caused by the *C. Ovivormes*; shows the arrangement of the growth, the condition of the epithelium, and numerous parasites enclosed in the cells, and lying free in spaces between the processes. (From a photograph magnified about 100.)

be repeated. Thus myriads of young sporozoa are soon found in the alimentary canal, the gall bladder, and the bile ducts of the infected animal. In this stage the organisms are very readily destroyed on being removed from the body, showing a marked difference, so far as resistance is concerned, when compared to the organism in its resting stage.

The sporules, on being set free from the mother cell, have the power of entering the epithelial cells of the affected region, where they commence a process of growth and differentiation of their protoplasm, which ends in the production of the encysted parasite. It is said that several parasites may infect the same epithelial cell, but, in the adult condition, one parasite is seen to occupy the greater part of the distal region of the cell body, and no trace of others can be seen. In course of time the epithelial cell wall is ruptured, and the parasite escapes, without necessarily causing destruction of the host cell; it passes through the alimentary canal, gains access to the atmosphere, and thus meets the conditions necessary for recommencing the cycle of development.

ANATOMICAL CHANGES PRODUCED IN THE HOST.

Let me now pass in review the changes produced by this parasite, whose life history I have described, and especially those in the liver where the disturbance is most obvious. The liver of an animal dead of the disease, after the acute stage has passed, or killed when the condition has become chronic, is studded over with greyish-white areas, varying in size from a pin's head to that of a pea, usually rounded, but occasionally somewhat branched in shape, resembling the aspect of chronic tubercle, a likeness which is more close as each spot of disease is filled with material which is apparently caseous. On examining these tumours, however, by means of microscopic sections, they are found to consist mainly of epithelium and connective tissue, which is arranged in the form of a very complex adenoma. The area of the bile duct affected becomes widened, and the space thus formed becomes filled up with the much hypertrophied and convoluted mucous membrane. The outer margin of the growth is marked off by a layer of well formed connective tissue, varying in thickness from the surrounding hepatic substance. Lining this fibrous layer is the epithelium

of the bile duct, which, in most places, has not preserved its typical cubical form, but is somewhat embryonic in character. The most striking feature of the tumour, however, is the extreme complexity of the processes projecting from the mucous coat. These projections are very finely branched, and thus the characteristic convoluted appearance is obtained. They carry with them a small quantity of fibrous tissue, and, usually, very large thin-walled vessels. Filling up the interstices of this adenomatous structure are seen multitudes of parasites in many stages of development. Some are encysted, and in this condition may be observed lying free, or still contained within the epithelial cells of the tumour. Naked



Fig. 49.—*a*, *b*, Coccidia occupying epithelial cells of the affected bile ducts; *c*, appearance presented after escape of the parasite.

forms may be seen from the smallest homogeneous globules of protoplasm, in all stages of granularity of protoplasm, according to their age, up to the adult form of the encysted parasite in which the protoplasmic contents of the cyst become very coarsely granular. These younger forms are seen usually lying embedded in the epithelial layer. If an animal still suffering from the acute form of the disease is examined, the crescentic spores, due to "endogenous sporulation," may be seen if special precautions are taken.

If the animal recovers from the disease, the tumour just described commences—apparently in a very short time—to alter its appearance. Many of the parasites pass out of the body, the remainder show signs of granular and fatty degeneration, their cyst walls resisting longest, and being, for some time, obviously

empty. The fibrous boundary of the adenoma becomes thicker, contracts, and, at length, the lesion caused by the attack of the parasite heals, and its site is marked by a small concentric nodule of fibrous tissue.

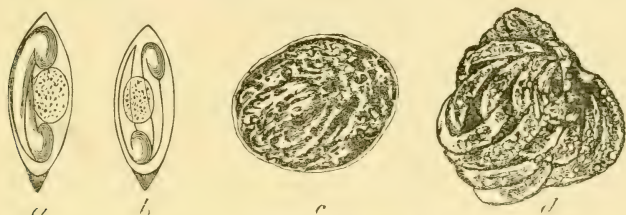


Fig. 50.—*a, b*, Formation of crescentic spores within the daughter sporophores, external to the host (after Balbiani); *c, d*, sporulation within the host, division of the spores into numerous crescentic segments. (After photographs by Pfeiffer magnified 1,000.)

In the small intestine, the areas of mucous membrane affected show similar changes to those described; the protozoa penetrate deeply into the glands, the epithelial cells become infected, the mucous membrane is thickened, and there results a hypertrophied, adenomatous condition of the particular area involved.

INOCULATION EXPERIMENTS.

Before passing from this division of my subject, I wish to draw attention to certain attempts which have been made to produce this disease by artificial introduction of the organism. Messrs. Ballance and Shattock attempted to infect certain animals by means of "psorospermial material," both by subcutaneous inoculation and intravenous injection.* Mr. D'Arcy Power, by means of specially-devised methods, has sought to produce the disease by the implantation of coccidia on specially irritated epithelial surfaces. Dr. R. Pfeiffer has taken coccidia which have passed through their cycle of development external to the body, and were thus presumably in a stage capable of causing infection, and injected them directly into the liver and into the veins of presumably healthy animals. All these experiments have yielded negative results, while the feeding of rabbits with ripe coccidia has brought about the disease in animals presumably healthy; and, so far as

* Ballance and Shattock: *Trans. Path. Soc. Lond.*, vol. xlii., p. 380.

we can judge, infection by the alimentary canal is probably the only method of infection.

CONCLUSIONS AS TO PROTOZOA IN RABBITS.

It may be taken as established in the case of the *Coccidium oviforme* attacking the rabbit that :

1. A most important portion of the developmental cycle of this parasite takes place only external to the body, under aërobic conditions.
2. Influences occurring outside the body delay, and even prevent the external sporulation of the parasite, thus interfering with its infective power.
3. The host cannot be infected by coccidia inoculated directly from animals already suffering, thus proving that the disease, though infectious, is so only in a very special way.
4. The parasite infects the host by passing into the alimentary canal, where it meets suitable conditions for its future development.
5. The parasite enters and grows within epithelial cells without necessarily destroying them, and causes great proliferation of the neighbouring epithelium.

THE QUESTION OF PARASITES IN CANCER.

Having drawn attention to the development of this well-recognised organism, and its effect on the tissues, I wish now to consider certain appearances which may be recognised in carcinomata, and to inquire what evidence there is of their parasitic character.

It is now more than forty years since Virchow* drew attention to the structure of certain cells occurring in tumours which appeared to contain inclusions of extraneous origin. At that time considerable discussion arose concerning these cell inclusions. The chief opinions then advanced in explanation of the bodies under observation were that they arose from the endogenous formation of cells, along with alteration of their nuclei, so as to give the appearance of homogeneous spheres (Virchow), or that they were due to the imbibition of albuminous or watery fluids, probably a part of a degenerative change (Henle, Bruch, and others).

*Virchow, *Arch. f. path. Anatomie*, etc., Bd. i, 1847, Taf. ii, Fig. 5, *k* and *l*, and Band iii.

The next series of researches of importance in the history of parasitic influences on cancer were undertaken as a result of the great additions which were made to our knowledge of pathological processes by the investigation of the pathogenic fungi.

After this period, the question of the parasitic origin of carcinoma seems to have been considered, in most quarters, as one of those which for all practical purposes was settled definitely in the negative. About the year 1888, however, the current of opinion began to change, taking a somewhat different channel. Increased attention was being paid to the lowest animal organisms, which had been long overlooked on account of the great interest which had been taken in the corresponding group of the vegetable kingdom, and the influence of these as possible factors in disease began to be more carefully studied.

On looking over the numerous papers written on this subject,* and especially by comparing the figures given by their authors, the conclusion which must inevitably be drawn is that the bodies described are of the most diverse character, and that undoubtedly many forms of cell and nuclear degeneration have been made to do duty as parasites, or are mentioned by the writers as having been described as parasites.

THE EXISTENCE OF A PARASITIC PROTOZON IN CARCINOMA.

Having accounted to myself for many of the peculiar structures described by various writers, there still remained one series of appearances which remained to be identified. I now refer to the cell inclusions which have been noted by various authors. After having investigated the matter for some time, and examined a large number of different cancers, I had become still more sceptical than at the outset of being able to identify any structure which might be considered with any degree of probability to be parasitic in character. When I was able to define this body, however, it became obvious that something totally different from the appearances already noted was under observation. Since that time I have examined a number of cancers from different regions—breast,

* For the bibliography of this subject refer to papers by Stroebe, *Centralblatt f. path. Anatomie u. allg Pathologie*, Bd. ii, 1891; Ruffer and Walker, *Journal of Pathology*, vol. i, 1892.

liver, alimentary tract, skin, etc.—and I am glad to say that I am able to corroborate most of the descriptions of the various writers.

The illustrations of this structure will be observed to be in many cases from cancer of the breast. What holds true about such typical growths as those of the mamma may be regarded as being also applicable to most other varieties of cancer.

DESCRIPTION OF THE PARASITE.

1.—*In the Cell Body.*—Taking, therefore, cancer of the breast as an example, if careful microscopic examination is made, there will be found lying, most commonly within the cell body, rounded or oval structures, varying in most cases from 2μ to 10μ in diameter, having, when large, a very distinct capsule, and presenting a smaller body of variable shape, situated, as a rule, towards the centre of the capsule. From the capsule there may be seen passing towards the centre numerous fine radial striations, and the capsule itself occasionally seems to have similar markings. On the other hand, passing outwards from the nucleus towards the periphery may be observed processes of a somewhat different character; they are not nearly so regular, and appear to be prolongations of the nucleus.



Fig. 51.—From cancer of the breast. Cells showing parasites contained in the cell body magnified about 800. *p*, Parasite; *n*, Nucleus.



Fig. 52.—Parasites enclosed within the cell body magnified about 800.

These bodies occur usually one in a cell, but there may be more; and, in some cases, eight or ten of small size may be seen

lying closely together in a cluster. In a successful preparation each of the small ones may be noticed to contain the usual nuclear substance.

2.—*In the Cell Nucleus.*—Similar structures of smaller size may be observed lying inside the nucleus of the epithelial cell. In this situation the capsule, which is so very characteristic of the intracellular inclusion, is very slight, and, indeed, appears to be absent in most cases. The nuclear inclusions may be single, or may also occur in small groups. Occasionally the bodies may be

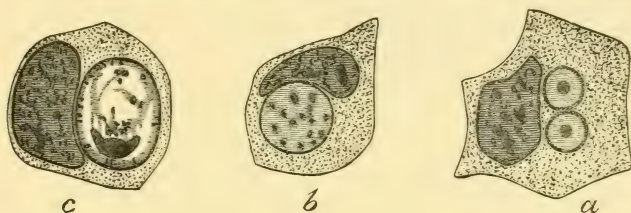


Fig. 53.—Cells from different cancers of the breast, showing various forms of parasites in the cell protoplasm magnified 1,200.

seen partly within and partly without the nucleus, as if they were passing out from the nucleus into the cell protoplasm. In this reference I would draw attention to an observation of Dr. Ruffer's* who has been able to show that, in certain cases, the nucleus seems to become filled up with numerous small parasites which escape into the cell protoplasm after having burst through the nucleus. The presence of the intranuclear forms does not seem to be so common as the intracellular variety, and, for some reason, they appear more plentiful in certain cancers than in others. The features shown by many preparations strongly favour the idea that in

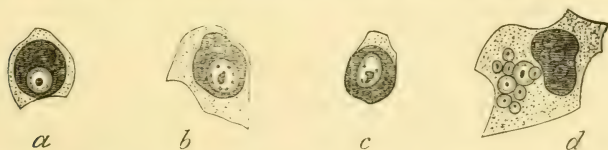


Fig. 54.—a, b, c, Cells showing single parasites occurring within the nucleus magnified about 800; d, numerous parasites in the nucleus and in the cell protoplasm magnified about 1,000.

* Ruffer, *British Medical Journal*, vol. ii., 1892, p. 993.

some cases the inclusions multiply readily within the nucleus, and ultimately free themselves from the nucleus and gain access to the surrounding protoplasm.

3.—*In the Intercellular Spaces.*—Bodies of similar character may be observed, in much smaller numbers, lying in the intercellular spaces, and more rarely still they may be seen lying two or three together in lines amongst the fibrous tissue at the margin of an alveolus, that is to say, in lymphatic vessels. It is difficult to say whether the latter appearances are accidental, but there seems to be no doubt that the bodies are of the same character as the intercellular and intranuclear forms already noted. They give the reaction which so readily distinguishes the body under consideration from the globules of altered chromatin which are so often seen in cancer and in other conditions, and with which, perhaps, they might be confused in unstained preparations.

4.—*Position in the Growth.*—The position of these bodies is a fact of important significance. They occur with greatest frequency in rapidly growing cancers, and in those cases where there is the least sign of cell degeneration. In the case of cancer of the breast they are found most numerous at the outer margin of the mass of cancer, or in the outlying alveoli, and in recently infected lymphatic glands. On approaching the centre of a mass of growth, or where degeneration has commenced, they begin to be less readily recognised, whereas the many varieties of so-called "cancer parasites" to which I have drawn attention become more and more numerous. The disappearance of these characteristic forms must not be taken as necessarily implying destruction, for it must be borne in mind how resistant are the adult coccidia in the case of the rabbit, and how they continue to develop in situations where their destruction by decomposition would seem inevitable.

It will be gathered from what I have said that I have described these bodies with so much detail for the purpose of concentrating special attention on them, as the only bodies yet found which show any probability of being parasitic. Their occurrence within the cell as a distinctly foreign substance, their appearance so strongly suggestive of an organised structure, the staining reactions which they give so distinct from those presented by the normal contents of cells, their great analogy in this latter respect, and especially in

their behaviour within the cell, and possibly also external to it, to well-known species of sporozoa recognized as parasitic in animals, all point forcibly to the conclusion that these bodies, though not necessarily coccidia, are nevertheless protozoa, and are parasitic in cancerous epithelium.



Fig. 55.—*a, b*, Groups of cells containing intracellular parasites magnified about 1,000; *c*, cancer alveolus from edge of rapidly growing carcinoma of breast, showing numerous parasites magnified about 400.

METHODS.

At an early period of this investigation it occurred very naturally to me that cancers had been probably the most frequently investigated, from the histological standpoint, of all abnormal tissues, and yet these bodies had not been described. I examined many old specimens of cancers prepared by myself in this laboratory for an investigation on totally different lines, and I also examined a number of excellent preparations kindly lent me by a friend, and prepared some years ago, when the question of parasitism was not being discussed; in none of them could I obtain satisfactory evidence of the parasite under consideration. The cancers from which the preparations were made were hardened by

immersion in Müller's fluid or chromic acid, and spirit. Being now accustomed to recognise this parasite, faint traces of them were observed in places, but nothing satisfactory nor convincing. The reason is, I believe, that the material was not properly prepared for the purpose.

Methods of Fixing.—The way in which I now attempt to proceed is to obtain the material as soon as possible from the operating table, fresh specimens being much more satisfactory to handle afterwards than material obtained from the *post-mortem* room. Pieces of small size ($\frac{1}{4}$ inch square) are then placed, one set in Flemming's fixing solution, and another set in corrosive sublimate, or in Foà's fixing solution. After remaining in the fixing solution for a sufficient time, the pieces of tissue are thoroughly washed, hardened in successive strengths of alcohol, embedded in paraffin, and cut in the usual manner.

Methods of Staining.—The staining reagents which have been found most useful are hæmatoxylin (Ehrlich) alone, or with some ground stain (rose bengale, eosin, etc.), and the Biondi triple stain. The reason for having the preparations fixed in two different ways is that Flemming's solution fixes the cell elements more sharply and definitely than any other not containing osmic acid, and if the parasites are present in the piece of tissue to be examined one feels sure of finding them. The staining reaction, however, with an osmic acid fixing reagent is not very brilliant, and for this reason it is well to have the other series of preparations at hand to obtain staining reactions if required.

Flemming's solution, followed by hæmatoxylin, gives, to my eyes, as good results in the way of sharpness of definition as can be obtained; the epithelial cell nuclei take up the hæmatoxylin in the normal way, but do not bear the same tone of blue as that shown by the parasites; in the case of the "Biondi" reagent—so strongly recommended by Dr. Ruffer and Mr. Walker—the distinctive colouring is much more striking and beautiful, and its results are well seen in the beautiful drawings accompanying their paper.

I would add, in reference to the matter of staining, that the secret of discovering the bodies is in the fixing and hardening of the preparations; no precaution should be thrown aside in carry-

ing out these processes. If this is well done, the observer need not seek far for stains if he wishes simply to see the parasite. A perfect differential stain to pick out the bodies alone is still to be found. The nuclear stains, such as methyl green, hæmatoxylin, are not very suitable for that purpose; like the coccidia in the rabbit's liver, the parasite seems to prefer general protoplasmic stains, and, acting on this principle, the best results of which I am aware have been obtained by Mr. Plimmer, who has used aniline blue as the special stain for the organism.

In all well-prepared specimens there is no difficulty in distinguishing the parasite from the ordinary forms of cell degeneration. It may be of interest to remark in this connection, that when the coccidia in the rabbit's liver were being first investigated, certain pathologists declared the appearances of this now well-known parasite as being given rise to by alterations in the surrounding liver tissue.*

CONCLUSION.

The further questions which arise—namely, can the parasites be observed “undergoing reproduction,” and can they be observed developing outside the body? What influence have they on epithelial cell multiplication? and, finally, have they a causative influence on cancer?—are now the points to be investigated; and it must not be considered a reproach if, as yet, no definite answer may be given. It will be remembered that, although the very characteristic *Coccidium oviforme* was recognised so long ago as the year 1839 by Hake in this country, it is only within the last few months that the description of the processes, apparently completing our knowledge of the anatomical changes in the life history of this organism and accounting for the enormous rapidity of its multiplication within the body of its host, have been made known by Dr. R. Pfeiffer.

* Lang, *Archiv. f. path. Anatom.*, etc., Bd. 44.

CELLULOID.—A new and rather surprising use, says *Anthony's Bulletin*, has been discovered and patented by Mr. C. H. Koyl, of Euston, Pa., for Celluloid. By silvering the back of a sheet of this material, Mr. Koyl has succeeded in producing a looking-glass which is not only of excellent quality, but is much less destructible, and has also the advantage of being bent or formed into any shape.

Prof. E. G. Balbiani's Researches on the Merotomy of Ciliated Infusorians.*

By FILANDRO VICENTINI, M.D. (Chieti, Italy).

PROF. Balbiani defines Merotomy as being the operation which consists in the separating or cutting from a living organism, a more or less considerable portion, for the purpose of observing the anatomical and physiological modifications of the isolated part.

In a previous paper † on the subject of artificial division, Prof. Balbiani traced the work of earlier writers, and gave a detailed account of the structure of *Cyrtostomum leucas*, and his study of the effects of merotomy on this species. He found that the merozoite, or fragment of the individual, which contained the nucleus, or a part of the nucleus, was alone capable of regeneration—namely, of constituting an individual similar, though smaller, to the original. Studies of *Trachelis ovum* and *Prorodon nivens* yielded essentially the same results.

In the present work, Prof. Balbiani describes some interesting experiments which he has made upon *Stentor cœruleus*. After comparing them with those of Gruber on the same species, he gives a detailed account of the various phases presented, from which we abstract the following :—

A.—Merotomy of *S. cœruleus* by artificial division.—In transverse sections intersecting the nuclear chain the anterior merozoite has to reproduce the rudder and the posterior sucker, but these are generated in less than twelve hours. The posterior merozoite has to reproduce a mouth, a peristome, and a new contractile vesicle. Should the *Stentor* be divided into three fragments, the middle one, containing a portion of the nuclear chain, regenerates in twenty-four hours the missing extremities, and the other portions behave as above. Should either of the pieces not contain a portion of the nucleus, it is not converted into a complete individual; but the protoplasm degenerates, becoming spongy, and dies in about twenty-four to forty-eight hours.

* *Annales de Micro.*, IV. (1892), pp. 396—407, 449—489 (3 plates).

† *Recueil Zool. Suisse*, V. (1888), pp. 1—72 (2 plates).

B.—Merotomy of Stentor by fission.—When the *Stentor* approaches the phase of fission, its nuclear chain shrinks up into a rounded mass. The author shows, first, the part allotted to the nucleus; and then that each offset resulting from the division of the primitive individual acquires by means of the plasm its own individuality before finally separating. He also finds that fission does not take place if each of the offsets do not contain a nucleus of its own.

C.—Merotomy of Stentor by Conjugation.—Stentors in conjugating unite together at the anterior extremities; the nuclei of the two individuals dissolve, and in their stead the micro-nuclei multiply by karyokinesis (or indirect division). On the separation of the two individuals a new nucleus and nuclear chain appear in each. The new nucleus * appears to be due to the blending of the micro-nuclear substances of the two individuals.

At the end of his paper Balbiani summarises his experiments and his opinions as to their results as follows:—

(1) The more or less large fragments which are separated from the body of a *Stentor* generally close quite easily the wound produced by the section. This closing takes place almost immediately owing to the contractility of the muscular fibres of myonemes (the fibres of Lieberkühn). When the wound is very large its closing is often incomplete, and imbibition is then probably delayed, as in the normal lacerations of the cuticle described by Schuberg, by the superficial coagulation of the denuded plasm.

(2) The local modifications of the wound and the contractions of the body which aid in closing it must be considered as phenomena of excitation determined by traumatism. This excitation can be observed also in the movements of locomotion, where they are manifested by a more rapid and less regular agitation of the vibratile cilia, as well as by a greater frequency of diastrophy or inversion of the normal direction of swimming.

(3) The phenomena of excitation may be observed as well in those merozoites containing nuclei as in those without any. After

* In his previous researches (1861—62) Balbiani regarded the nucleus as an ovarium and the nucleoli as a testis. (On the conjugation of the infusoria and other relative questions, see Carpenter, "The Microscope and its Revelations," 7th edition, by Dallinger, 1891, pp. 709—712.)

the period of excitation—which is generally of short duration—and when the laws of equilibrium are not too profoundly disturbed, the fragments regain their normal orientation and regularity of movement—behaving, in a word, like ordinary Stentors.

(4) The most apparent and remarkable phenomenon of merotomy is the rapid and complete regeneration of those merozoites which contain the whole or part of a nucleus, a single segment of the nuclear chain being enough to induce regeneration as rapidly and completely as that performed by the entire nucleus. Regeneration is generally completed in about twenty-four hours, varying according to the temperature.

(5) Should the peristome be removed, it is re-formed by a rudiment which, as in reproduction by division, appears first in that part of the ventral surface to which Schuberg has given the name of the branching zone (*Verästelungszone*). The new peristome is completed by a mouth and an adoral zone, which are also formed as in the process of division. The contractile vesicle is not reproduced as a new organic formation of the plasm, but by a simple local dilatation of the previous excretory system. The reconstitution of the nuclear chain is the last act in the regeneration of the merozoite. It takes place by successive divisions of the nuclear particles which the merozoite first contained. The new particles having the same volume as the primitive particles, it results that the nuclein increases at the expense of the plasm. The regeneration of the merozoite is sometimes followed by a tendency to multiplication by division; in other words, to a second reproduction of new parts. But these are soon reabsorbed, and the individual reappears in its original aspect. This phenomenon is probably caused by a superabundant physiological activity of the nucleus induced by the lesion.

(6) The merozoites which do not contain any part of the nucleus never form a complete individual. They present a short period of excitation, which is manifested in the same way as in those containing a nucleus. When a merozoite without a nucleus contains a mouth or anus, it ingests food or rejects the undigested masses like a normal individual. The nucleus is thus shown to have no influence over the ingestion or rejection of food. Merozoites without a nucleus do not survive more than from twenty-four

to forty-eight hours at most ; the cause of death is the alteration of the plasm, which presents a vacuolated or spongy aspect owing to aqueous imbibition, and probably also to the arrest of the functions of assimilation. The principal seat of aqueous imbibition is probably the wound, owing to its not being completely closed. The same cause which prevents the reproduction of the lost parts prevents also the disappearance of the irregularities of form which are often the consequence of traumatism and which give rise to the merozoite. These irregularities fully disappear in the course of regeneration among the nucleated merozoites.

(7) The opinion of Gruber that the nucleus is necessary to give an impulse to the formation of new organs and useless for the further development of these organs is inexact. The presence of the nucleus is indispensable at all stages in the formation of the organs.

(8) When a merozoite without a nucleus has been taken from an individual which is preparing for spontaneous division, but has not yet shown any sign of outward contraction, the division of the merozoite takes place as if it continued to form part of the intact individual ; but, whilst this would have given birth to two complete buds, the merozoite produces only portions of the two buds which would have formed at the expense of the mass of plasm which composed it.

The division is but rarely carried to a complete separation of the two parts. As soon as it arrives at a certain stage, a retrograde movement takes place, and the two parts are again mingled in a common mass, which is destroyed by degeneration. When one of the two parts is only a miniature of the other, it sometimes becomes completely independent, but soon perishes. It is to be inferred from this observation that the impulse which determines the division of the plasm comes from the plasm itself and not from the nucleus, but that the nucleus is necessary to sustain this impulsion and carry the division to the end.

(9) The micro-nucleus, whether alone or accompanied by the nucleus, takes no part in the regeneration or other vital manifestation of the plasm. Its object is to intervene in the phenomenon of conjugation ; it is, to use Bütschli's expression, a sexual nucleus (*Geschlechtsken*).

(10) The non-intervention of the micro-nucleus in the vital phenomenon of the plasm is also proved by the fact that it has no influence over the intracellular absorption of the parts of the old nucleus at the time of conjugation. It is only when by its fusion with the congeneric element of another individual thus becoming a truly active nucleus, that it effects the absorption of the old nucleus. This absorption, which has a great analogy with the digestion and assimilation of food, is probably due to a secretion having its seat in the plasm, and which is dependent on the nucleus as in the other secretions of the Protozoa.

(11) The fragments which have been separated by artificial division from a *Stentor* in the state of conjugation are regenerated when the segments of the nucleus which these fragments contain present a clear and homogeneous appearance—a sign of their vitality. In the more advanced stages when these segments appear grey and granular—a sign of their approaching disorganisation—regeneration does not take place, but the fragments containing them degenerate and die. But these fragments regain the power of regeneration when the new nucleus makes its appearance in the plasm and exercises its functions as an active element, as previously described.

Prof. Balbiani is of the opinion that the physiological influence of conjugation is clearly shown by these experiments.

It is related by the British Consul at Cadiz, Spain, in illustration of the perfection with which natural wine can be imitated by modern chemical methods, that he and a friend, visiting one of the native sherry cellars there, were given two samples of wine to drink, which seemed to be almost identical; and when told that one was a natural product, and very costly (250 dols., equal to £50, a bottle), while the other was a manufactured product, the market price of which was only a few cents a bottle. In making the imitation, the natural product is first analysed, and the chemist, ascertaining the exact nature of its constituent parts, is able to combine them, and thus nearly reproduce the original compound.

Why should not Injected Material be Hardened as carefully as Ordinary Material?

By PROF. V. A. LATHAM, D.D.S., Chicago University.

IT is time to consider the vast importance attached at the present time to micro work, that greater attention should be paid to the matter of hardening. Why this is so badly attended to is scarcely to be understood. If it *can* be neglected without detriment, why need we trouble to do it at all? To this many will answer, why, to keep the parts in a good condition. Then is this true where the hardening is not *thoroughly* done? We aim to preserve the cells and tissues in as *natural* a state as possible, to keep the tissue as near as possible to the original condition, to prevent shrinkage of the elements; and here we require every care, so as to be able, in pathological growths, to distinguish the particular kinds of neoplasm, as, for example, a large or small round-celled sarcoma, or a spindle-celled from a round-celled. These are important, and if badly hardened the spindle-cells shrink so as to make them nearly round in appearance. Injected material is useless for good histological or pathological work, if not hardened well.

A slide should show the cell and tissue-structure quite as clearly as the course of the blood-vessels, lymphatics, or bile-ducts, for otherwise we do not get the correct idea of their relationship to the cells, etc. To-day the idea of a slide to show injections, and another for the structure, is out of place, when they both can be so well done. Alcohol is only permissible as a hardening agent in very small, thin pieces of tissue, where rapid examination for diagnosis is desired, and here very great care should be used to have the alcohol in varied strengths, using a weak solution for a few hours, a stronger for a little longer time, until complete hardening is done in strong alcohol. The abstraction of the water from the tissues by the alcohol, and its power of coagulation, rendered it a little dangerous for hardening for diagnosis, or for preserving micro-organisms in the tissues. Chromic acid, 1/6th per cent. (2 parts of solution of acid and 1 of spirit, stir), as advised by Klein, is decidedly one of the best and most reliable agents known, but it has its objections—that the

material must be cut into small cubes, and its power of rendering micro-organisms *unstainable*. For objects in bulk, as amputations, small or large animals, if an opening be made in the abdomen and thorax, so the fluid will penetrate, Müller is the best agent, and it does not interfere with the staining of micro organisms. Its objections are the length of time it takes, and the slight colouring of the tissues by the potassium bichromate, though this, by frequent washing with running water, can be aided, previous to the tissue being put into weak spirit to the strong, or by a 5 per cent. solution of hydrate of chloral. Chromo-osmic acetic acid is also good for small tissues.

The main point to be remembered is, we require *to fix the elements as soon after death as possible, by an agent which will not contract the tissues too suddenly, by abstracting the water too quickly and coagulating the albumen*. These methods of hardening are the best also for injected tissues, if *used ice cold* when the gelatin masses are employed. I find a good way is to use the solution of Müller to keep the animal in whilst injecting with hot masses, as gelatin, instead of the ordinary hot water.

Why Leaves Change Colour.

A BOTANIST has thus explained in *Forest and Stream* why leaves change colour:—The green matter in the tissues of a leaf is composed of two colours—red and blue. When the sap ceases to flow in the fall and the natural growth of the tree ceases, oxidation of the tissue takes place. Under certain conditions, the green of the leaf changes to red; under different conditions, it takes on a yellow or brown tint. This difference in colour is due to the difference in combination of the original constituents of the green tissue and to the varying conditions of climate, exposure, and soil. A dry, cold climate produces more brilliant foliage than one that is damp and warm. This is the reason that the American autumns are so much more gorgeous than those of England.

There are several things about leaves that even science cannot explain. For instance, why one of two trees growing side by side, of the same age and having the same exposure, should take on a brilliant red in the fall and the other should turn yellow; or why one branch of a tree should be highly coloured and the rest of the tree have only a yellow tint, are questions which are as impossible to answer as why one member of a family should be perfectly healthy and another sickly.

Note on *Lavatera Arborea* (The Tree Mallow).

BY R. LAWTON ROBERTS, M.D.

I HAVE received from a friend in the Channel Islands a section of a stem of the Tree Mallow (*Lavatera Arborea*), and it is of such prodigious dimensions that the plant from which it was cut must have been quite exceptional in point of size.

Turning to the third edition of Sowerby's "Botany" (edited by Syme), I find that the "stem" of *Lavatera Arborea* is stated to be "woody, 2 to 8 feet high, and in large examples often 1 inch in diameter." (The italics are my own.)

Now, I have kept my bit of stem for some months, so some shrinkage has taken place, yet I find it measures over 5 inches in diameter in one direction and 4 inches in another, and a good 15 inches in circumference. I am informed that the girth of the fresh stem was uniformly 16 inches or just over, up to a point 3 feet from the ground, where it branched.

The history of this giant amongst mallows is soon told. A Mr. Dancaister erected for himself a dwelling near the shore of St. Owen's Bay, Jersey; and in September, 1891, this gentleman cleared a small portion of the adjacent ground. Soon afterwards he noticed a number of seedlings of *Lavatera Arborea* shooting up, and, as the ground was not required for some months, the young plants were left unmolested. On October 10th, 1892, when my friend visited the spot, a little forest of monster tree mallows had sprung up; but of these luxuriantly growing plants, which numbered over a score, only one had attained the enormous dimensions already described.

"The height of the main stem," writes my friend, "was 3 feet and the girth *uniformly* 16 inches or just over to the point whence it branched. It was a fine plant for one of the *Malvaceæ*, the topmost leaves being quite 8 feet high."

The rapidity of growth of this specimen seems remarkable, since the unusual girth of the stem was attained in thirteen months from the time the plant first showed as a seedling.

The Tree Mallow—though usually considered rare—grows

freely in Jersey; and, since the usual habitat of the plant is found to be "maritime rocks," it is probable that the greater fertility of the cleared soil near the shore of St. Owen's Bay was the cause of the excessive luxuriance and size of the samples of *Lavatera Arborea* seen by my friend on October 10th, 1892.

Microscopical Technique.

COMPILED BY W. H. B.

Substitute for Glass for Covers and Slides.*—In using Celluloid—viz., wood rendered soluble in ether and alcohol with gum camphor—for films for microphotography, Dr. Edwards was struck with the idea that it could be used in microscopy. It is much cheaper than glass, and almost as transparent. Being unbreakable and very light, it is especially valuable for sending by post. It is stronger than wood, has no fibre, and can be cut readily with scissors. It can be obtained with a ground surface as well as plain. The thin celluloid films commonly used for instantaneous coverers can be employed for covers, whilst the thicker kind used in ordinary photography makes capital slides. Dr. Edwards has some an inch square, and mounts them temporarily on a glass slide for use on the microscope.

Preparing *Artemia fertilis*.†—Dr. J. E. Talmage finds that the mounting of *Artemia fertilis*, the brine shrimp, is by no means a simple undertaking; most of the ordinary media either causes the delicate structure to become distorted or produces such a transparency as renders the whole object invisible. The method which he now uses is to mount them in a preparation of the lake water (the lake from which he gathers his specimens is the Great Salt Lake, Utah), with corrosive sublimate and an alcoholic solution of carbolic acid. The living *Artemiæ* are placed direct into this fluid; they die quickly, in so doing spreading themselves out most perfectly. Before mounting, he makes a very shallow cell of hot paraffin and balsam, and after the cover-glass is in position he

* *Microscopical Notes*, by Dr. A. M. Edwards, Newark, N.Y., U.S.A.

† *The Microscope*, XII. (1892), pp. 238—240.

rings the edge with a very little of the same material, following this with repeated layers of cement (King's preferred).

Examination of the Blood of Amphibia.*—Herr M. C. Dekhuyzen uses test-tubes holding 8 ccm. and having a diameter of 14 mm. These are placed in a wooden stand and filled with the fixation fluid or with simple salt solution. In the latter case they are filled first with water and boiled, and the slides are also treated in the same way; the cover-glasses are cleared with acetic acid and water, and, after drying, with ether. The two fluids used were—(a) [1] A 2 per cent. solution of osmic acid, [2] 6 per cent. acetic acid containing 24 per cent. of a watery solution of methylen-blue, and a little (0·014 per cent.) acid fuchsin; (b) the other fluid contained 20 volumes of acetic acid mixed with 80 volumes of methylen-blue solution, 6 volumes of this fluid mixed with 14 volumes of 1/5th per cent. solution of acid fuchsin gave the required concentration.

Before every fixation 2 ccm. of the last deep-blue mixture was well mixed with 6 ccm. of 2 per cent. osmic acid and placed in small tubes, which were filled up to the top.

It is important to be very careful in allowing the blood when it comes from the blood-vessels to come into the most intimate contact with the fixing mixture. The blood-cells sink to the bottom. After thirty minutes a drop of the fluid should be placed on a slide, and then some of the bottom be drawn up and added to it; the cover-glass should be run round with xylol balsam. The preparations must be kept from the light.

Sterilising Incoagulable Albumen.†—M. E. Marchal suggests that the action of certain salts may be utilised to prevent the coagulation of egg-albumen when heated to 100 deg. These salts are borate of soda, sulphate of iron, and nitrate of urea. The following are the quantities of these substances to be used for the purpose:—Solutions of 2 to 5 per cent. :—Borate of soda, 0·05 gm. per litre; sulphate of iron, 0·001—0·006 gm. per litre. Solutions of

* *Journ. R. Mic. Soc.*, 1893, p. 116; from the *Verhandl. Anat. Gesell.*, 1892, pp. 90—93.

† *Journ. R. Micr. Soc.*, 1893, p. 112; from the *Bull. Acad. Roy. Sci. de Belgique*, XXIV. (1892), pp. 323—27.

10 per cent. :—Nitrate of urea, 4 to 5 grm. per litre, Thus prepared, the liquids may be sterilised at 100 deg. in cultivation flasks.

It is hardly necessary to point out that nitrate of urea should not be used to prevent the coagulation of albumen if the experiments relate to nutrition or fermentation of matter containing albumen.

Preparing and Staining Yeast.*—For fixing preparations of yeast, Dr. H. Moeller uses a 1 per cent. solution of iodide of potassium saturated with iodine, this fluid ten times diluted, and also iodine-water. The material and the fixative may be mixed together at once or upon the cover-glass, which merely requires a smear. When fixed and dried the preparation must be thoroughly hardened. This may be done by leaving the preparations in the iodine solution for a day, and then, after washing in water and weak spirit, keeping them in absolute alcohol for one or two days. The time required for hardening may be diminished by repeatedly boiling the alcohol, and the preparations are more clearly stained if they are then immersed in chloroform for a day. It is always useful to pass the cover-glasses once or twice through the flame.

The preparations are best stained by means of hæmatein and picric acid, the latter acting as a mordant. But it is essential that the preparations should be thoroughly fixed and hardened; they may then be treated with a saturated aqueous solution of picric acid for from half-an-hour to three hours. The preparation is then passed through water so as to wash off some, but not all, of the picric acid. For staining, an alkaline solution of hæmatoxylin is used. It would not appear, however, that the foregoing staining was more advantageous than that with aniline, of which the following were successfully employed :—Phenol-fuchsin, alkaline, methylen-blue, Gram's method, and also gentian-violet in carbolic acid, water, glycerin, 1 per cent. acetic acid, and 1 per cent. iodide of potash.

If the anilin dyes are used, the preparation should be overstained and then differentiated by some decolorant; if Gram's method be adopted, alcohol must be used; but for other stains a

**Journ. R. Micr. Soc.*, 1893, pp. 118—119; from the *Centralb. f. Bakteriöl. u. Parasitenk.*, XII. (1892), pp. 537—50.

mixture of equal volumes of glycerin and water was found to give the best result. As soon as the desired degree of decolorisation is attained, the preparation is washed in water, dried in the air, and mounted in balsam, styrax, or dammar.

The grana or microsomes were best brought out by staining with some anilin dye and then differentiating with 2 per cent. acetic acid.

Spores are very easily stained by treating the preparation with boiling phenol-fuchsin and then washing out in 4 per cent. sulphuric acid.

The yeasts used for these observations were natural cultivations of ordinary bottom yeasts. The yeast was shaken up with distilled water, and then, after settling, the fluid decanted off. The sediment, after having been thus treated several times, was kept for observations.

Minute Structure of Butter.*—J. Ferdinand points out that, when microscopically examined, the fat-cells of pure butter appear round and regular. Granular masses of casein and albuminous matters may be seen in addition if the butter has not been prepared carefully, and occasionally spores or filaments of penicillium are present under these circumstances. Added animal fat (as in margarine) shows crystals, either separate or in groups. The polariscope and selenite plate are of use in examining these.

Aniline Dyes as Antiseptics.†—Dr. C. Prioux points out that solutions of pyoktanin and gentian violet in the strength of 1:100 prevent the development of micro-organisms. Weaker solutions (1:500 and even 1:2000) arrest the cultures of the typhoid bacillus and *B. coli communis*. One per cent. solutions of safranin hinder the development of Eberth's bacillus, and it is maintained that the violet anilines constitute the most powerful antiseptics.

Glycerine Jelly for Mounting.‡—The difficulty of satisfactorily enclosing micro-mounts prepared with pure glycerine has induced

* *Journal d'Hygiene.*

† *Merck's Bulletin.*

‡ *Pharmaceutical Journal.*

many workers to substitute glycerine jelly wherever practicable. Many formulæ have been published for this medium, but one recently published by J. E. Huber possesses a degree of novelty in that he excludes the use of water as one of the ingredients. He recommends that clear gelatine (1 drachm) be allowed to macerate in glycerine (1½ oz. by weight) overnight, and that the mixture should then be heated in a water-bath until solution is perfect. Specimens to be mounted should be soaked first in dilute, then in stronger glycerine, afterwards placed on a slide with as little dilute glycerine as possible, and covered with hot jelly. After cooling, the cover-glass is placed over the object, heat applied to the slide, and the cover pressed down into position when the jelly melts. On cooling overnight the slide may be cleaned and finished off. The mounts are stated to be free from the liability to shrinkage that often occurs when glycerine jelly is used.

Notes on Bone Technique.—In the section “Microscopy” in the *American Naturalist* for June, 1892, Dr. C. O. Whitman gives the following notes for preparing sections of bone:—“In preparing bones for sectioning, it is well to have fresh material taken from a young individual. After the soft parts are removed, the bone is cut into short pieces, and then macerated in water until the medulla is easily washed out. They are then ready to section.

Preparations nearly as good as those obtained by maceration may be made from fresh tissue. Thin sections are cut from the desired region with a fine saw. From these the medulla should be carefully washed out under a jet of water; they are then ground until the desired thinness is reached, again washed, dried, and mounted. The grinding may be done with a file or on a revolving grindstone, or with emery on a dentist's lathe,* or between pieces of compact pumice stone, followed by hones of finer grain, and finally polished on a piece of smooth leather or buckskin, covered with powdered chalk.

Another method is to grind the bone on a glass plate with emery of different degrees of fineness. This may be accomplished by pressing the section down with the fingers, or it may be fastened to a cork by means of sealing-wax or thick balsam. It is then polished on one side until smooth; then the wax of balsam

* Healey, *Amer. Mon. Micro. Journ.*, 1884.

is melted, the section turned and polished on the other side until the required thinness is reached. Only compact tissue can be prepared by this method. The spongy tissue, being delicate, must be embedded before sectioning. This may be done according to the method given by Wiel,* Koch's copal method,† or a mixture of ten parts resin and one of ordinary wax may be used.‡ The objects should be placed in a very fluid, but not too hot, solution of the above, and after a short time lifted out with forceps, leaving as much of the mixture as possible adhering to the object. When cool, the mass may be cut into thin sections and ground in the ordinary way, washed and cleaned in turpentine, and mounted in balsam. If an opaque preparation be desired, the embedding mass is removed by washing in chloroform and the sections dried between sheets of filter-paper and mounted.

A very convenient method is given by Ranvier.§ The fragment of bone is placed in a syrupy solution of gum arabic, and when saturated it is exposed to the air until the gum thickens; it is then hardened in alcohol. From this mass sections are made and ground in the usual way, except that alcohol is used to wet the bone instead of water. When ground sufficiently thin, the gum is dissolved in water and the section is ready to mount. According to the method of mounting, either opaque or transparent preparations are made. For the study of Haversian canals, lacunæ, and canaliculi, the former is better. To obtain an opaque preparation, a drop of balsam is placed on the slide and heated over a spirit-lamp to evaporate the oil. It is then cooled and tested by a needle. If hard, the balsam is again softened and the dry section placed in it; at the same time a drop of balsam is placed on the cover-glass, which is applied, and the whole transferred to a cold surface. This should be done as quickly as possible, in order that the balsam may solidify before penetrating the cavities. If, on the other hand, we wish to study osseous lamellæ as stained preparations, the section is first placed in a solvent of balsam, then transferred to a warm solution of balsam until the entire canalicular system is filled, when it is mounted."

* *Zeit. f. Wiss. Mikros.*, Bd. IV., p. 200, 1888.

† Whitman's *Embryological Methods*, p. 233.

‡ Ehrenbaum, *Zeit. f. Wiss. Mikros.*, Bd. I., p. 14, 1884. § *Traité*, p. 249.

Notes.

DR. A. Rothpletz (*Botanisches Centralblatt*, 1892, pages 265—68) advances an interesting theory as to the formation of oolite. An examination of calcareous material from the shore of the Great Salt Lake, Utah, U.S.A., revealed the fact that they were covered with a bluish-green coating, which consisted of colonies of the lime-secreting algæ, *Glæocapsa* and *Glæotheca*. The lime enclosed by the alga is in roundish masses and is of a finely granular texture. Dr. Rothpletz says they are undoubtedly produced by these algæ. He has also investigated the oolitic bodies from the shores of the Red Sea, and believes that they are produced by similar algæ. Analogous structures are described from the limestone of the Vilser Alps. The author is of the opinion that the greater number of the marine calcareous oolites with a regularly zoned and radial structures are produced by these algæ.

Apropos of the Great Salt Lake, Dr. J. E. Talmage, in the *Microscope* for December last, disposes of the assertion, often made, that no living thing can exist in its waters. He records the presence of four forms:—1, *Artemia fertilis*, Verrill, abounding in large numbers; 2, Larvæ of one of the Tipulidæ, probably *Chironomus oceanicus* (Packard); 3, A species of *Lorixa*, probably *C. decolar* (Uhler); and lastly, the larvæ and pupæ of a fly, *Ephydra gracilis* (Packard). He also considers that the “vegetable life of the lake is a subject worthy of investigation.”

Hitherto the two groups of *Macro-* and *Micro-lepidoptera* into which butterflies and moths have been divided have been characterised by the former including all the large and conspicuous species, and the latter only containing small and inconspicuous moths. In a recent communication to the Entomological Society of London, Dr. Chapman has endeavoured to raise the Micros in general favour by transferring to that group several of our finest moths. According to him, the pupa of the Goat-Moth (*Cossus ligniperda*) possesses all the characteristics of a typical micro-lepidopterous pupa, and for a similar reason the genera *Sesia*, *Zygæna*, *Procris*, and *Hepialus* ought to be placed among the micros. The loss of the pretty burnets, clearwings, and foresters will hardly be welcome news to the macro-lepidopterist, nor will the disturbance of a well-arranged drawer of micros, to make room for the large and chronically “greasy” goat and swift moths, be considered an altogether favourable change. Still, other elements besides the

structure of the pupa must necessarily be taken into consideration before a satisfactory system of classification is arrived at, and Dr. Chapman's views will probably afford material for considerable discussion. It is not so many years since the genus *Psyche* was removed from the Bombyces (where it formed a striking contrast to the giant *Saturnias*) and placed in the micro-lepidoptera.

Mr. T. Mellard Reade, in the *Geological Magazine* for March, supplies the latest computation as to the age of the earth. He says :—"The mean area of denudation throughout post-Archæan times being taken as one-third the entire land-areas of the globe, the bulk of the post-Archæan rocks being expressed by the land area of the globe two miles thick, and the rate of denudation one foot in 3,000 years ; the time of accumulation will be $5280 \times 2 \times 3000 \times 3 = 95,040,000$. *The time that has elapsed since the commencement of the Cambrian is, therefore, in round figures 95 millions of years.*" The italics are Mr. Reade's.

At the February meeting of the R.M.S., the Rev. Dr. W. H. Dallinger, in discussing Mr. Nelson's paper on the chromatic curves of microscope lenses, pointed out that unless some means could be devised to allow of the employment of the shorter wave-lengths of the spectrum, we had nearly reached the limits of visual possibility with the means at present at our disposal. He also considered that there could be little doubt that all who believed in the advantage of monochromatic light foresaw that there must be lenses specially prepared for its use.

ELECTRIC LIGHT AND PLANT STRUCTURE.—G. Bonnier has conducted some interesting experiments to ascertain how the structure of herbaceous plants is influenced by exposure to the electric light. He finds that direct electric light is prejudicial to the normal development of the tissues on account of its ultra-violet rays. Generally, when considerable development, accompanied by intensification of the green colouration, is caused by continuous electric light, in plants growing under glass shades that intercept excess of ultra-violet radiation, at first high differentiation occurs in the structure of the organs ; but an intense light, prolonged unchanged for months, causes remarkable modifications of structure in the various tissues of such new organs as are capable of adapting themselves to the illumination. Less differentiation then takes place in these organs, though they are always rich in chlorophyll.—*Comptes Rendes*.

LAO TEA.—According to the *Kew Bulletin*, the Laos, who live in the neighbourhood of Chiengunai, Siam, do not use tea-leaves for preparing infusions, but prepare them for chewing. The leaves of the *Camellia theifera* are steamed, then tied up in bundles, and buried in the ground for about fifteen days. The product is termed “mieng,” and is said to keep for two years or more. It is reported to be almost indispensable to natives engaged in hard work.

POTATO DISEASES.—A disease in potatoes has made its appearance in several districts in the Bengal Presidency, which is quite distinct from that caused by the *Phytophthora infestans*. It is now in course of being investigated locally, but Mr. G. Massee is of opinion that it closely resembles the disease caused by *Peronospora trichotoma* on *Colocasia antiquorum* (an edible corm or Yam) in Jamaica.—*Kew Bulletin*.

AMBERGRIS.—H. Beauregard is of opinion that ambergris may be considered as an amber-coloured calculus containing a proportion of black pigment and some excrementitious matters. Pieces extracted from the intestines of the sperm whale appear to be formed by an aggregation of acicular crystals arranged in different positions. If examined under the microscope, with the aid of polarised light, these crystals are readily differentiated from the surrounding mass by the brilliant colours displayed on revolving the prism, and it is suggested that the peculiarities of structure disclosed should be utilised for the rapid investigation of samples suspected to be adulterated.—*Journal de Pharmacy*.

Correspondence.

Will some microscopist kindly give me a little information respecting the mounting of spread diatoms? I find, after acid treating and washing the material, that when put on the slide with balsam there is such a cloudiness that spoils the appearance. I shall be glad to know the cause and the cure; so will other friends, I presume, as I have received several in exchanges with the same objection.—JOHN T. NEEVE.

PLANT HAIRS.—Examine the hairs from the flower-stalk of the *Cypripedium*, scrape a few off the stem, and place them under a 1/5th in. objective, 1 in. o.c., and also with polariscope. They are most interesting.
E. S. MATTISON, M.D.

WEAK SOLUTIONS.—Is it possible that solutions of 0.0001 per cent., if used for five minutes, are of any use in staining goblet cells, etc.?
E. P.

Beautiful Slides of Marine Algæ, with reproductive organs, etc., in exchange for other Slides, Materials, Books, or Accessories.—JOHN T. NEEVE, 68 High Street, Deal.

Twenty-four Microscopic Slides, various, offered in exchange for either of the following books :—Gray's "Marine Algæ," Pennington's "Zoophytes," Hooker's "Student's British Flora," John's "Flowers of the Field," Prantl and Vine's "Botany," Spencer's "Biology," etc.—J. T. NEEVE, 68 High Street, Deal.

Reviews.

THE WORKS OF HUBERT HOWE BANCROFT. Vol. 38, Essays and Miscellany. 8vo, pp. vi.—764. (San Francisco: The History Co.; London: Trübner and Co. 1890.)

This volume contains accounts of Early American Chroniclers; The New Civilisation; Root-diggers and Gold-diggers; Treatment of the American Races; and many other important papers by Mr. Bancroft, which had not previously been published.

THE WORKS OF HUBERT HOWE BANCROFT. Vol. 39, Literary Industries. 8vo, pp. vii.—808. (San Francisco: The History Publishing Co. London: Trübner and Co. 1890.)

This, the concluding volume of the series of Mr. Bancroft's gigantic work, gives a history of the author's labours. It explains his methods and tells of his trials and triumphs. Over thirty years ago he commenced the task, which was accomplished on the completion of this volume, during the whole of which time he tells us his efforts have been continuous. Sickness and death have made their presence felt, but he never lost interest in his work or felt it irksome, and all who read his books cannot fail to be assured of the fact that from first to last his labour has been a labour of love. Of all the books which it has been our privilege to read during the last twelve or thirteen years none have afforded us more real pleasure, and it is with feelings of no little regret that we are compelled to look on this as the last of the series. One of his reviewers has well said, "Your work will remain to coming ages a treasure-house of information."

THE FIELD CLUB. Edited by Rev. Theodore Wood, F.L.S. Vol. 3. 8vo, pp. 194. (London: Elliot Stock.)

This is a magazine of General Natural History for Scientific and Unscientific Readers, and contains a great number of interesting articles relating to all departments of Natural History.

AN ACCOUNT OF BRITISH FLIES (Diptera). By Fred. V. Theobald, B.A., F.E.S. Vol. I. 8vo, pp. xx.—215. (London: Elliot Stock. 1892.)

Perhaps no order of insects is more common than Diptera and no order is less understood. This is doubtless due in a great measure to the scarcity of literature on the subject. The book before us comes, therefore, very opportunely. It commences with a short account of the more important character-

istics of the families of Diptera, followed by chapters on Fossil Diptera; the Classification of Diptera; the Aphaniptera; the Cecidomyidæ; the Mycetophilidæ; the Bibionidæ and Simulidæ; and the Chironomidæ. The book is published in bi-monthly parts at 1s.

TEXT-BOOK OF BIOLOGY. By H. G. Wells, B.Sc.Lond., F.Z.S. With an Introduction by G. B. Howes, F.L.S., F.Z.S. Part I., Vertebrata. Cr. 8vo, pp. x.—149. (London: W. B. Clive and Co. 1892.) Price 6s. 6d.

This little book—which is one of the Univ. Corr. Coll. Tutorial Series—contains in a concise form a large amount of useful information, the subjects treated being the Rabbit, Frog, Dog-Fish, and Amphioxus, followed by a chapter on Development, Miscellaneous Questions, etc. We are disappointed with the plates which are diagrammatic, the reference-letters being very indistinct. The student, however, will find the book helpful.

THE BUILDING OF THE BRITISH ISLES: A Study in Geological Evolution. By A. J. Jukes-Browne, B.A., F.G.S., etc. Second edition, revised. Cr. 8vo, pp. xii.—465. (London: George Bell and Sons. 1892.) Price 7s. 6d.

The aim of the book before us is the restoration of the physical and geographical conditions which prevailed in the British area during each of the great periods of time which make up our geological sequence, the author's chief object being to trace out the succession of physical and geographical changes which have led up to the existing disposition of land and water in the north-western portion of Europe. There are 15 plates, chiefly diagrammatic.

ELEMENTARY MATHEMATICAL ASTRONOMY, with Examples and Examination Papers. By C. W. C. Barlow, M.A., B.Sc., and G. H. Bryan, M.A. Cr. 8vo, pp. vi.—442. (London: Clive & Co. 1893.) Price 6s. 6d.

In the book before us—which is now in its second edition—the aim of the authors has been to produce a book that shall take its stand between the popular and non-mathematical treatises, and those which involve high mathematics, and to prove a suitable text-book for such examinations as those for B.A. and B.Sc. of the University of London. The diagrams are clear and good.

ZOOLOGY FOR SECONDARY SCHOOLS. By C. de Montmahon and H. Beauregard; translated by Wm. H. Greene, M.D. Cr. 8vo, pp. 368. (Philadelphia: J. B. Lippincott and Co. 1893.)

This book forms the basis of instruction upon the natural history of animals in the secondary schools of France, and treats the subject in a manner found by experience to excite most interest on the part of the pupil. The illustrations, 319 in number, form an important feature of the work, and are the best of the kind we have seen.

HANDBOOK OF PRACTICAL BOTANY. By E. Strasburger, edited from the German by W. Hillhouse, M.A., F.L.S., etc. Third edition. 8vo, pp. xxiv.—425. (London: Swan Sonnenschein and Co. 1893.) Price 9s.

A very desirable work for the microscopical botanist and all those who wish to become acquainted with the elements of scientific structural botany. It is divided into thirty chapters, leading the student on by easy stages. The first assumes on the part of the worker entire ignorance as to the use of his instruments. In the appendices are given an alphabetical list of plants used for study; an alphabetical list of reagents, and how to prepare and use them; and Notes on Methods and Selected Reagents. There are upwards of 100 very clear and good illustrations.

THE FIELD NATURALIST'S HANDBOOK. By the late Rev. J. G. Wood and the Rev. Theodore Wood. Fifth edition. Cr. 8vo, pp. 167. (London: Cassell and Co. 1893.) Price 2s. 6d.

A most excellent book for the field naturalist; its scope is confined principally to Entomology, Field Botany, and Egg-Collecting. Each month in the year is taken separately, a complete catalogue being given of all the butterflies and moths which appear in it, together with the plants in flower and their localities. In addition to each insect, there will be found notes on its eggs, caterpillar, and pupa. The food plant is also given. At the end of the entomological portion we find a chapter describing the localities most frequented by each species of butterflies and moths, and the best methods of taking them. Birds are classed according to their order, beginning with hawks and ending with petrels.

There are also short chapters on Breeding from the egg, larva, and pupa, with full details of the best modes of catching, setting, and preserving butterflies and moths, and of blowing and preserving birds' eggs, and drying and arranging plants. A young naturalist cannot afford to be without this book.

THE YEAR-BOOK OF SCIENCE. Edited for 1892 by Prof. T. G. Bonney, D.Sc., LL.D., F.R.S., etc. Cr. 8vo, pp. viii.—519. (London: Cassell and Co. 1893.) Price 7s. 6d.

This is the second year of the publication of this useful book, which gives a report of the scientific work of the past year. It will be noticed that more attention than in last year's volume has been given to Zoology. Thus we find Animal Biology divided into the following sections:—Zoology and Comparative Anatomy; Animal Physiology and Pathology; and Bacteriology. Botanical Biology treats of Systematic and Geographical Botany; Morphology and Biology of Plants; Minute Anatomy of Plants; and Physiology of Plants.

MORE ABOUT WILD NATURE. By Mrs. Brightwen; with illustrations by the Author. Crown 8vo, pp. xvi.—261. (London: T. Fisher Unwin. 1892.) Price 3s. 6d.

We are charmed with Mrs. Brightwen's book. She is a thorough lover of animals, and has given us some very interesting sketches of their habits. There are also hints on Home-museums and for studying living insects and how to keep them as pets.

HAZELL'S ANNUAL for 1893. Cr. 8vo, pp. 740. (London: Hazell, Watson, and Viney. 1893.) Price 3s. 6d.

This is truly a cyclopædic record of men and topics of the day, giving an account of the year's history in all parts of the globe, revised to Nov. 30, 1892. Amongst the new subjects discussed in the present volume are articles on Bimetallism, about 26 Biographies, Building Societies, Chicago's World's Fair, The General Election of 1892, Political Parties, Uganda, etc. etc. A most useful book.

OUR SECRET FRIENDS AND FOES. By Percy Faraday Frankland, Ph.D., B.Sc., F.R.S., etc. Foolscap 8vo, pp. 167. (London: Society for Promoting Christian Knowledge. 1893.) Price 2s. 6d.

This, which is one of the "Romance of Science" Series, will do much towards making the reader more intimately acquainted with the low forms of germs or micro-organisms now attracting so much public attention. It treats of micro-organisms in the air and in water, useful and malignant micro-organisms, and the theory and practice of prevention in disease. There are nearly 50 good illustrations.

A MANUAL OF BACTERIOLOGY. By A. B. Griffiths, Ph.D., F.R.S.E., etc. Cr. 8vo, pp. xv.—348. (London: W. Heinemann. 1893.) Price 7s. 6d.

Those desirous of knowing something of the Science of Bacteriology will do well to study this one of Heinemann's Scientific Handbooks, which treats of the Methods of Cultivating, Staining, and Mounting Microbes; their Origin, Classification, and Identification; the Biology of Microbes; Microbes of the Air, Soil, Water, etc.; with many other particulars. There are 56 illustrations.

BACTERIOLOGICAL DIAGNOSIS. By James Eisenberg, M.D.; translated by Norval H. Pierce, M.D. 8vo, pp. xiv.—184. (London: F. A. Davis and Co. 1892.) Price 8s. 6d.

A series of Tabular Aids for use in practical work in the Study of Bacteria, in which 138 micro-organisms are considered in the following order:—I., Non-Pathogenic Bacteria—*a*, Liquefying Gelatin; *b*, Non-Liquefying Gelatin. II.—Pathogenic Bacteria—*a*, Cultivated outside the animal body; *b*, Not Cultivated outside the animal body. III.—Fungi. We are much pleased with the tabular arrangement, an entire page being devoted to each species. Here is given the specific characteristics of the various well-established bacteria, so that the worker may at a glance inform himself as to the identity of a given organism. These Tables are followed by an Appendix, in which is given Microscopical Technique used in the cultivation and staining of Bacteria; a Laboratory Inventory; and a good Index. It is unquestionably a most useful book.

THE MEDICAL ANNUAL and Practitioner's Index. Cr. 8vo, pp. lx.—644. (Bristol: John Wright and Co. London: Simpkin, Marshall, Hamilton, and Co. 1893.) Price 7s. 6d.

We have received the eleventh Annual Volume of this important work. It contains a report of the progress of Medical Science in all parts of the world, together with a number of original articles. There are eight plates and about eighty wood engravings. The volume is in every respect equal to its predecessors.

CHOLERA; Its Protean Aspect and its Management. By Dr. G. Archie Stockwell, F.Z.S. In two vols. Vol. I. Fscap. 4to, pp. vii.—132. (Detroit, Mich., U.S.A.: G. S. Davis.) Price—2s. cloth; 1s. paper covers.

The author positively denies the assertion that Cholera and several other diseases "are diseases whose microbic origin is positively known," but believes that if immunity is to be secured it will only be at the price of "eternal vigilance," coupled with more perfect knowledge. This is one of the well-known Physician's Leisure-Hour Series.

NOTE-BOOK FOR DENTAL STUDENTS. By James F. Rymer. Second edition. Foolscap 8vo, pp. 67. (London: C. Ash and Sons, Broad St., Golden Square. 1892.)

We think this book will prove helpful to students. The information contained in so small a compass is necessarily of a condensed nature. The book is interleaved throughout with blank paper.

ELEMENTS OF HUMAN PHYSIOLOGY. By Ernest H. Starling, M.D.Lond., M.R.C.P. Foolscap 8vo, pp. 464. (London: J. and A. Churchill. 1892.)

In this little book will be found very clearly and concisely expressed such of the main facts of Physiology as are of importance to the student. There are nearly 100 diagrammatic and other illustrations.

EARLY HISTORY OF THE RETREAT, YORK: Its objects and influence. By D. Hach Tuke, M.D., LL.D. 8vo, pp. 96. (London: J. and A. Churchill. 1892.)

A paper on REFORM IN THE TREATMENT OF THE INSANE, read at the Centennial Meeting of the Retreat, held at the Institution, May 16th, 1892. With a report of the celebration of the Centenary.

HOW NATURE CURES, comprising a new system of Hygiene; also the Natural Food of Man. By Ernest Densmore, M.D. 8vo, pp. 413. (London: Swan Sonnenschein and Co.) Price 7s. 6d.

A statement of the principal arguments against the use of bread, cereals, pulses, potatoes, and all other starch foods. The author says, in the future, when the doctrines herein taught are understood and adopted, mankind will become "fruitarians." A fruit diet means the solution of the problems of how to banish disease and intemperance from the race, and to give us a food which is, at once, in accord with our higher instincts and the demands of æsthetics.

A MANUAL OF CURRENT SHORTHAND, Orthographic and Phonetic. By Henry Sweet, M.A., Ph.D., LL.D. Crown 8vo, pp. xx.—137. (Oxford: The Clarendon Press. 1892.) Price 4s. 6d.

In this system the author claims to give us a system of writing shorter and more compact than ordinary longhand, and, at the same time, not less distinct and legible. By this system the two styles, orthographic and phonetic, can be written concurrently, so that orthographically written words, *e.g.*, proper names, etc., can be inserted in a phonetically written passage without confusion. We could have wished that the alphabet, and some of the earlier examples, had been written in much larger characters.

A SYNOPSIS OF TRIGONOMETRY. By William Briggs, B.A., LL.B., F.C.S., etc. Crown 8vo, pp. 43. (London: W. B. Clive & Co.)

One of the Univ. Corr. Coll. Tutorial Series. A very concise and useful little book.

STANDARD ARITHMETIC. By William J. Milne, Ph.D., LL.D. Crown 8vo, pp. 428. (New York: American Book Co.)

In many cases in the book before us we think the rules, and explanatory reasons for working the different problems, are more plainly expressed than in some of our own arithmetics.

CASELL'S NEW TECHNICAL EDUCATOR, Parts 4 and 5, monthly. Price 6d.

This is an excellent periodical for home study. The contents of each number are very varied, and treat of Steel and Iron, Plumbing, Cotton Spinning, Projection, Cutting Tools, Drawing for Carpenters and Joiners, Photography, The Steam-Engine, Watch and Clock Making, Electrical Engineering, etc.

RECORDS OF THE PAST. Vol. VI. Edited by A. H. Sayce, Hon. LL.D. Dublin, Hon. D.D. Edinburgh. Crown 8vo, pp. xxii.—160. (London: S. Bagster and Sons.)

This volume completes the New Series of Records, which are English translations of the Ancient Monuments of Egypt and Western Asia, and contains, amongst other interesting papers, Historical Inscriptions of Rameses III.; List of Places in Northern Syria and Palestine conquered by Ramses II. and Ramses III.; Letters from Phœnicia to the King of Egypt in the 15th Century, B.C.; The Non-Semitic Version of the Creation Story, etc., etc.

THE STORY OF PAUL BOYTON. Crown 8vo, pp. viii.—358. (London : George Routledge and Sons. 1893.)

This is a most entertaining book, giving an account of voyages on all the great rivers of the world, paddling over twenty-five thousand miles in a rubber dress. He gives a number of thrilling experiences in distant lands among strange people. We were so interested in this book that we could not leave it till we got to the end. A splendid book for boys, whether young or old.

THE MARVELS OF THE POLAR WORLD. Translated from the French by Robert Routledge, B.Sc. Lond., F.C.S., etc. Post 8vo, pp. 256. (London : George Routledge and Sons.) Price 1s.

One of "Every Boy's Library" series, giving interesting accounts of the Polar world, its seasons, flora, fauna, the inhabitants of the extreme north, etc. There are 38 good illustrations. This is just the book to interest boys.

PHOTOGRAPHS OF THE YEAR. (London : Hazell, Watson, and Co.) Price 10s. 6d.

Consisting of 12 beautiful Glypogravure pictures, suitable for, and worthy of, framing, accompanied by descriptive notes and critical review of the Photographic Society's Exhibition, 1892. These pictures are by far the best we have seen.

THE LONDON STEREOSCOPIC and Photographic Co., Ltd., have sent us specimens of the OWL NOTE PAPER. Each sheet has a picture, on top right hand corner, of a pair of remarkable species of Australian birds, *Podargus Strigoides*, which migrates to, and breeds in, the British Islands during the summer months. These birds have been sketched from life in various attitudes by W. Saville-Kent, F.L.S., F.Z.S., who has had a pair in his possession for three years. The sketches, although true to nature, are most comic and amusing.

THE YEAR-BOOK OF PHOTOGRAPHY for 1893. Edited by T. C. Hepworth, F.C.S. Crown 8vo. (London : Alexander and Shephard.) Price 1s. 6d.

This is a book of about 650 pages, of which about 300 form the Year-book, which contains the results of Photographic Experimenting during the past year, and many special articles by leading photographic authorities; with formulæ, details of processes, useful tables, and many illustrations. The Year-Book keeps up its standard of excellence.

BRITISH JOURNAL PHOTOGRAPHIC ALMANACK. Edited by J. Traill Taylor. 1893. (London : Henry Greenwood and Co.) Price 1s.

Here is a book of nearly 1250 pages, of which about 500 represent the Almanack, which, as usual, is full of useful information, giving the results of the experiments and researches of the last year. There are also a number of good plates and other illustrations.

HANDBUCH DER PHOTOGRAPHIE. By Dr. Josef Maria Eder. Nos. 20, 21, 22, 23. (Halle a S. : Wilhelm Knapp.)

In these four numbers of Dr. Eder's Photographic Handbook we have a description of the various lenses employed in photography and the methods to be employed in testing them.

BY THE SEA and other Poems. By Fred. Henderson. Second Edition. Crown 8vo., pp. 78. (London : T. Fisher Unwin.) Price 2s 6d.

Remarks on some of the Phases seen in a few Organisms found in Decomposing Blood, etc.

BY R. L. MADDOX, M.D., HON. FELL. R.M.S., etc.



HAVING requested the butcher to supply me with some blood from his slaughter-house for use as manure, I received a very large pailful of blood, seriously contaminated by some garbage, which, as far as I could see, was mostly composed of the entrails of ducks and fowls, all being in the early stage of putrefaction. Before making use of the material supplied, it occurred to me that it might be worth while to examine the more fluid part with the microscope. Upon so doing I was struck with the multiplicity of the various organisms present, consisting chiefly of different bacteria, bacilli, spirilla, though rare, and a few very arched vibrios, with some very minute flagellate infusoria, besides numerous cell-structures, with duplicate nuclei.

There was one point, however, that seemed to me rather peculiar about some of the large rod bacilli, and which, though possibly common, I had never before noticed so distinctly, and which is best described as being of a horse-whip character or shape. This was seen not only in the long filaments, but also in the short ones, though a great many, *apparently* of the same kind, had the width equal throughout the full length. The material was found so exceedingly dirty, it seemed useless to attempt to make any serviceable slides. It was, therefore, put aside in the room and kept lightly covered from dust and light, as I was desirous more especially to note the further character of the rod bacilli. No attempt was made to procure a pure culture by plate cultivation for separating the different organisms, as I preferred to keep the material as received.

The fluid was re-examined after a couple of days, when much of the *débris* had sunk to the bottom. The same objects were now seen more clearly, and I was enabled to mount a slide showing many of the general features. As all were not found in the

same field, only one has been selected, the chief objects being the putrefactive bacteria, slender bacilli in chains, two of the stout rods somewhat narrowed at one end, a spirillum, and a curved chain of possibly lactic acid elements, which is seen somewhat out of focus at the edge of the circle in Photograph No. 1 (Fig. 56).

Upon continuing the examination a day later, a considerable change was noticed; the putrefactive bacteria were not so numerous. The rods of all lengths were plentiful, of equal width, and noticed to have bluntly rounded ends. The whip-



Fig. 56.—A few of the organisms found in the material when received :—Bacteria, bacilli, two whip-shape rods, a spirillum, and lactic ferment, $\times 520$.

shaped rods were not quite as abundant as the others, but many of them were seen with the thick ends discharging their spores, and with spore formation occurring in nearly the whole of the remaining length of the rod. These points are shown in the photographs, Figs. 57—58, though many of the rods of both kinds extended far beyond the field of the microscope when using the $1/5$ th objective and No. 1 ocular.

The spirilla, which had been somewhat sparse, appeared at



Fig. 57.—Rods of equal width ; in length many were treble the diameter of the field, $\times 520$.



Fig. 58.—A whip-shape rod filled with and discharging spores at the thick end, $\times 520$.

the next day's examination to be more numerous, though chiefly as free commas or single joints scattered loosely and generally immobile. The size varied from almost straight, short rods to the full-size curved joint, some having, as is very commonly the case, the curves opposed, others with the curve turned the contrary way or assuming the spiral shape. In some of the free joints one end could be seen to be *slightly* larger than the other. Although diligent search was made for growing spores, I was not able to conclusively satisfy myself upon this point; but in photograph, Fig. 59, such a condition as stated is to be seen



Fig. 59.—A few free commas or joints of spirillum before formation of colonies, $\times 520$.

in the central comma in the field, where the appearance is that of an oval enlargement, placed rather irregularly at one end, and a somewhat similar state is seen in one or two of the others. Scattered about in the field, though infrequent, well-defined round or rather oval bodies were noticed, which I concluded were the spores of the spirillum, and not of the long rods, which appeared to be a trifle larger. Amongst the few commas that were mobile, it was most difficult, after numerous methods of

staining had been tried, to find one with a single flagellum.

The next examination, a day later, offered for view quite a different set of objects, or rather similar objects in a different stage. The spirilla were now found in numerous small and large colonies, varying from five or six organisms to more than a hundred gathered together. Lying amongst them were, here and there, seen the small circular or oval bodies which I regard as spores—some free and some apparently attached to one end of a few of the felled commas. These immobile organisms, though not measured, appeared a trifle larger than the free ones previ-

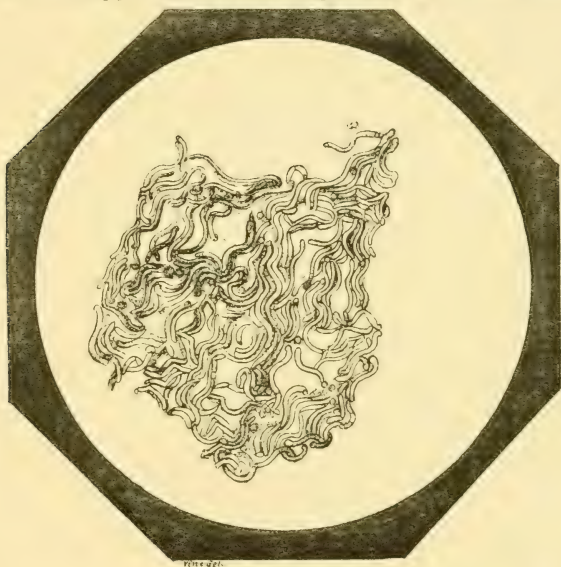


Fig. 60.—A spirillum colony with a few free spores, $\times 520$.

ously described. In some of the colonies, at the border, a few could be seen separated from the general collection, and in one or two cases with a *single* flagellum. Whether these had divided off from others, and so produced the flagellum at the point of separation by drawing out the inner plasma, or any living plastic membrane, or whether they had grown with the growth of the organism, I will not venture to decide. As I have several times seen, in the full-grown spirillum with the joints adhering, projecting from the points where separation into commas would take

place if the organism broke up into its components, a flagellum at either side alternately, I am inclined to suppose a flagellum can be formed at one end of the original comma, though I do not state it positively, because flagella can often be seen lying about broken off, and then, of course, such a feature may be accidental. One of these colonies is shown in the photograph, Fig. 60, and if carefully examined some of the points indicated can be readily seen.

On resuming the examination the next day, the free commas or joints were found to be far more numerous than on the day



Fig. 61.—Free commas, in greater abundance, after the formation of the colonies, $\times 520$.

prior to the appearance of the abundant colonies, but very few of them had divided. Nearly all were now mobile. In a few of them could be seen the oval or round bodies, apparently attached or lying close against one end of the joint. Three of the small bodies can be seen free, placed almost in a line towards the centre of the field in the photograph, Fig. 61, where also the other point stated is indicated. Part of the field is somewhat out of focus, which was due to the preparation being tilted by some dirt out

of the true plane. Upon close examination, some of the commas may be noticed as having a slight enlargement at each end. Whether this is caused by the ends only of the little curve being in contact with the cover-glass, and thus not in correct focus with the other part, it is difficult to state.

Upon resuming the microscopical examination two days later the whole material appeared to be charged with very active flagellate infusoria, from very minute ones, which appeared when seen edgewise as rather narrowed and beaked, through all sizes up to the large ones, which were provided with minute cilia

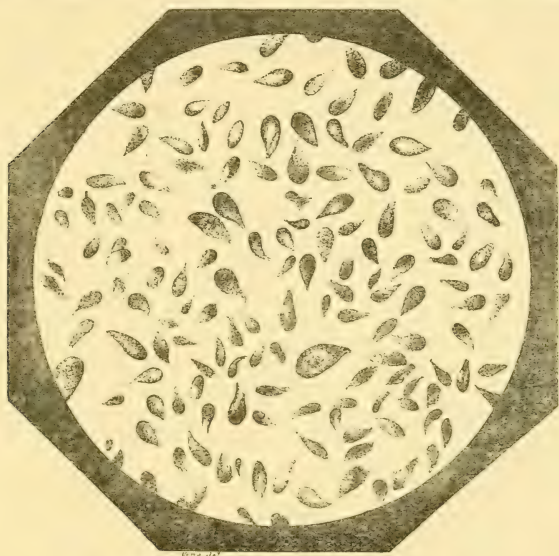


Fig. 62.—Flagellate Infusoria, $\times 200$.

covering the entire body, and at one end with a long pendant cilium. Scarcely any other organism could be seen in the examination of many fields. Unfortunately, the method employed to fix and stain them much altered their shape by contracting the contents of the body. Nevertheless, a preparation was made and a slide mounted with xylol balsam. Some apology is needed for the indistinct outline and detail of these objects, as represented in the photograph, Fig. 62. Several attempts were made to procure a better negative, but I found I had to content myself with

being satisfied by only a general focus, the objects varying so much in size and photographing badly. The main point, however, is well indicated; that is, their extraordinary abundance. It would have been better to have preserved them in some neutral medium as solution of ammonia chromate, after staining by a weak solution of logwood, or else to have killed them by a solution of chloral hydrate and mounted in weak potassic acetate solution. In most cases it is exceedingly difficult to so apportion the specific gravity or strength of the solutions that contraction or swelling shall not occur at the same time that the objects are preserved. Many of the solutions useful for other objects often cause a coarse granulation to appear with considerable cloudiness of structure. Being little interested at the moment in more than the numbers present, the subject was not pursued.

The examination was continued on the following day, and proved, perhaps, the most interesting, though the previous condition of the material led me to fear it would prove wholly futile. I was considerably surprised to find an almost total absence of the infusoria, and in their place scattered over the field numerous active free commas and some consisting of two joints, with others of several turns. In a fair number of those which might be called mature, and in others consisting of the free joints, a differentiation of the internal, usually homogeneous plasm could be most beautifully seen, especially after the treatment, which will be immediately noticed.

The change varied from trivial shading, and passed into a more or less distinct location at sundry points, leaving small spots or clear spaces very distinctly indicated, some of these being circular, others rather oval, and placed longwise across the breadth of the rod. From two to four could be made out in many of the single joints and from three to four in what would be each joint in the main growth or mature spirillum. These I regard—though I avoid making the assertion—as spores, not vacuoles, and think they go far to support the statements recorded by the celebrated microscopist, Dr. Henri van Heurck, when photographing the commas or joints of the so-termed cholera bacilli with an objective having the largest angular aperture yet constructed, N.A. 1.6, the objects being mounted in styrax with a very high

refractive index, and (as required to obtain the greatest advantage the use of the objective offers) upon a flint glass slide and cover. (*Vide* a letter from Dr. van Heurck, with figures from his photo-micrographs, which unfortunately do not do justice to his work, in the *English Mechanic*, Oct. 7, 1892.)

The preparation from which my photograph, Fig. 63, is made is of a wet one :—A saturated solution of potassic acetate and distilled water, equal parts ; while the photo-micrograph was made with an old Gundlach immersion, $1/15$ th, and which was employed for all the photographs except No. 7 (Fig. 62)

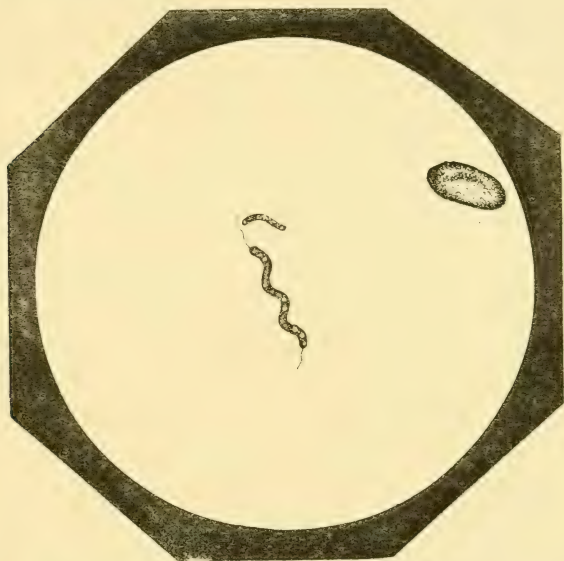


Fig. 63.—A spirillum and free joint, showing flagella and changes, with spores that have occurred in the plasm, $\times 520$.

Upon close examination, I at last found a field of view in which both the mature spirillum, with its flagellum at each end, and a separate joint lying near, each showing the differentiation that has been alluded to in such a position that both could be photographed at the same time. For many years, or at least since making my experiments on the organisms found in the excrement of the domestic goat and goose,* I have been exceed-

* *Vide Journal R.M.S.*, Dec., 1882.

ingly interested in the structure of spirillum, and have cultivated those found in horse-dung and in duck-droppings in many ways, yet had never arrived to such a stage as to obtain these demarcations so clearly. There is a point in these observations that is rather curious, which is the revival, so to speak, of the spirilla after the almost sudden appearance and abundance of the infusoria, of which only a *very few* were then present, and those of the minutest. Upon further keeping of the material there was no indication of the presence of any of the spirilla, the changes had become so complete as to be inimical to life.

To make this article a little more complete, it may be as well to state some of the various methods adopted and found most useful in the many examinations. In those cases where the objects in the blood-fluid were not allowed to dry direct on the cover-glass, it was diluted with an equal amount of pure water for after-treatment, and this always on the cover-glass. A very thin ring of Hollis' liquid glue, weakened by wood naphtha, was struck on the slide; a droplet of water was then put on the clean cover-glass; and then an equal portion of the fluid material, taken from just beneath the surface, was mixed with it, or rather placed on it; a thin platinum wire, twisted into a loop about the diameter of $1/16$ th of an inch to the $1/14$ th (as two sizes were kept at hand), then fixed by melting into the end of a glass rod for a handle, and which I have used for years for the same purpose, enabled me to apportion the amount of fluid on the cover so that it did not run beyond the ringed space. After waiting a short time for the objects to settle more or less on the cover, by means of a pair of weak forceps, it was turned over on to the centre of the ringed spot and allowed to fall gently. It was then examined under the microscope, using the $1/5$ th objective with No. 1 ocular, and if sufficiently clean and worth the trouble it was further dealt with to fix the objects in position. This was generally effected by placing a small drop of a saturated solution of tannic acid in water at one part of the edge of the cover, at the same time placing the point of a strip of thick blotting-paper at the opposite side. This effected a suction of the tannin under the cover-glass and across the objects; if the little stream appeared to flow too violently or too much in one

direction, then the blotting-paper was shifted. Sometimes two or three little strips were used to divert the flow, so that the minute free organisms should not be withdrawn out of the ring. After the tannin had acted for five minutes, a drop of water was placed at the edge, and this sucked through, repeating its use as often as appeared necessary. When the tannin solution had been removed and the washing made effective, a drop of a saturated solution of sulphate of iron with 10 grs. of citric acid to the ounce of solution were allowed to act for five minutes, and then washed out by water as described for the tannin; then the mounting medium was placed at the edge of the cover, and by means of absorbent paper made to take the place of the water. A solution of chrysoidine was sometimes employed after the water washing, if the sharpness of the outline seemed deficient in the objects.

The same method was adopted when using any of the ordinary aniline stains, besides employing the usual plan of drying the material on the cover, then using the different fixing or staining materials, and washing from a pipette, or by soaking, allowing the cover to float on the water. Some of the minute dirt-particles I found were sucked out to the advantage of the mount, and no doubt some of the loose organisms went with the dirt. It took some time to accomplish before the cover could be fixed down by the usual Hollis' glue, but I found the plan under the circumstances to yield me the best effects. The tannin, as is well known, has an immediate action on such parts of the organism as the cilia and flagellum, and also tends to fix the soft plasm; the iron solution, after its action for a short time, stains both parts of the organism, showing a pale grey tint in the flagella, which readily catches the eye when using the microscope. Logwood solution and iron were also tried, and likewise reversing the solution of tannin and iron by allowing the iron to act first, but preference was given to using the tannin first. All this detail will no doubt be sadly wearying to those who are far more efficient workers than myself; they will therefore excuse it, I trust, in favour of those less experienced than themselves.

The foregoing will show that in the original material supplied to me, I had, although in an early stage of putrefaction, what

turned out to be a good culture medium, and which permitted of following several of the interesting phases of growth and change without more than ordinary trouble. The temperature which for one part of the day was at summer heat in my room, may have had much to do with these changes.

It would be impossible to say from whence the rods both of equal width and whip shape, and the spirilla, were derived ; but, from my examinations of the excrement of the duck a few years since, I am strongly inclined to suppose they each were derived from it as left in the entrails thrown into the pail of blood, which itself, no doubt, was of a rather heterogeneous character. But little attention was paid to any of the other organisms. I will not attempt to classify either the rods or the spirillum, which might be *Spirillum undula*, as this is found in putrefying ditch water. The rods are larger than those of the hay bacillus, as found in infusions of hay or grass, and none of the spores were found growing like the spores of the hay bacillus. As to the more or less whip-shaped rods, I can offer no suggestion. The food material of both the duck and fowl partaking of both the animal and vegetable kinds, it would be difficult to fix the source without considerable trouble and favourable opportunity. Sufficient has, however, I trust, been indicated to show that our knowledge of these minute organisms is far from perfect, yet every fragment has its value. It may, perhaps, be worth while to state that when examining the long, largely-arched vibrios with well curved ends, I was surprised to see one suddenly spring back like a well-bent bow to instantly resume its place, as if in effort to free itself, though it remained otherwise motionless. This occurred twice in different individuals.

All the photo-micrographs were taken with the same objective, and the objects magnified 520 diameters, except that of No. 7, Fig. 62, which was taken with the 1/5th objective, and magnified 200 diameters.

On the Cultivation of Diatoms by Artificial Means.

BY DR. P. MIQUEL. (Translated from *La Diatomiste*.)

CHAPTER II.

GROWTH OF UNMIXED DIATOMS.

WE may consider two methods for the cultivation of unmixed diatoms. The first is one in which one kind only grows and multiplies, to the exclusion of all other siliceous algæ; the second is one in which one special diatom is evolved to the exclusion of all other living organisms, whether infusoria, green algæ, fungi, bacteria, etc. This latter should be called the cultivation of Diatoms *in a state of absolute purity*.

If the first of these methods is comparatively easy of success, the second is, on the contrary, very difficult, which arises, not from the impossibility which is often found of separating the diatoms from the green algæ, the fungi, and the protozoa, but from the bacteria that often live as parasites on the exterior thallus of the frustules.

I.—GROWTH OF ONE SINGLE SPECIES.

In order to be assured that you have in any maceration but one species of Diatoms, you may use several processes—some special and not equally applicable to all species; others are general, and furnish in all cases certain results if they are applied with judgment.

Special process.—I suppose that it is desired to insulate from a natural or artificial growth, containing numerous species, a filamentous diatom of the family of Melosiras. If the filaments of *Melosira* rise in the liquid above the sediment—which is very often the case—you take hold with tweezers, whose tips have been previously heated, a portion of the filament, which may be visible to the naked eye or may require a lens, and wash it repeatedly in sterilised water, and then quickly, and before it dries, place it in a fresh maceration that has been maintained for a quarter of an hour at 70°C. and then cooled.

The washing is intended to detach from the filament, as far as is possible, any other Diatoms that may be adherent to it; it will not always succeed, as you often find other species grow by the

side of the filament that has been selected. Nevertheless, by this first action, you have eliminated many species and have established the predominance of the *Melosiras* in the preparation, which, in contact with a new and specially suitable environment, will attain in a few days a superb development, furnishing tresses of filaments which rise in the liquid, and which allow you to recommence the process, with the probability of insulating the *Melosiras* absolutely.

It is equally easy, with a little practice, to separate, under a low-power microscope, *Fragilarias*, *Diatomas*, *Biddulphias*, etc., grouped in longer or shorter chains, and to place them in the nutritive liquids. But this operation is infinitely more difficult when it is desired to separate *one* living Diatom. We may say, without exaggeration, that every frustule seized by the tweezers is a broken frustule and therefore incapable of reproduction.

If the diatoms could be previously reduced to a dry state, the difficulty that I have noted would be easily overcome. However it may be, I attribute my failure solely to want of skill in withdrawing from a maceration, either with a capillary tube, a bristle, or the point of a forcep, any *small* diatoms previously determined on. Success is a little more certain with the larger kinds, such as the *Coscinodisci* and other species, that have a diameter or length of a tenth of a millimetre. In this case they may be insulated on the stage of the microscope, and by a bristle conducted into drops of distilled water in series on the mounts, and finally on to a piece of cover-glass placed in a new maceration.

Observers who are in this way able to insulate with certainty diatoms of all sizes will have no need to recur to the "general" processes, which require much longer manipulations.

GENERAL PROCESS FOR THE SEPARATION OF DIATOMS.

The process that has hitherto given me the best results is that which depends on the division—*fractionnement*—of a previously arranged culture. It consists in putting the diatoms in suspension in such a volume of water that 5 ccm. of that water shall enclose at least one frustule, which results in this, that when you sow 1 ccm. of the dilution in five macerations, you will have four sterile and one fruitful. This method requires a preliminary experiment.

Preliminary Experiment.—The diatomaceous sediment is agitated in a small bulk of sterilised water, and this water—still turbid, but deprived of the larger impurities by decantation—is placed in a new flask, or one that has to be heated almost to redness.

A drop of this liquid is placed on a slip of glass, whose upper surface is divided into squares of one-tenth of a millimetre. The water is allowed to evaporate, and you count under the microscope how many diatoms are held in suspension in each evaporated drop, noting especially the number of the frustules of the species that you desire to insulate.

Let us suppose that the drop has deposited five hundred frustules on the surface of the lined piece of glass—four hundred of various species and one hundred individuals of the species you desire to insulate. Hence, it is nearly certain that one drop of this liquid would introduce into a litre of water five hundred frustules—one for 2 ccm.

The result stated :—Thus, by introducing one drop of this tested liquid into a litre of water, and distributing half-a-ccm. of this water (after well shaking it) into twenty new macerations, five among them will have been fertilised with one diatom and fifteen will remain sterile. In the five cases of fecundation, the operator may hope for a pure culture of the diatom he seeks to insulate.

Results.—After having exposed to the light of day the macerations thus treated, it will be found that things are as above stated, that twenty-five for every hundred cultures will give positive results ; in a word, experiment will prove the theory. But the observer may simplify this work, and put on his side all the chances of success. He should endeavour to secure in the cultures the predominance of that diatom that he desires to obtain in a state of purity. In point of fact, if this diatom is found in the primitive mixture in the proportion of four hundred to five hundred ; in five cases of fecundation, four will be determined by the species that he desires to insulate, and then in place of twenty macerations it will suffice to put a dozen to work.

The common risk in this method is the fear of not pushing the dilution to a degree sufficiently advanced ; and on the other side it is hardly necessary to add that if the dilution be too great the results will be negative, whence the need for the *preliminary experiment*.

When the microscope shows that the species is in a state of purity—that is to say, without admixture with any other diatoms—you may proceed in order to ensure success to a new dilution less rigorous and only with half-a-dozen macerations. This second operation is intended to convert into certainty the probability already given by the microscopic examination. For even when in a microscopic preparation you can only find diatoms of one kind, it is possible that the macerations may contain others, which, at first very rare, ultimately multiply and become frequent. Therefore, I consider it always indispensable to take a second dilution and a second division with the cultures that are supposed pure.

I ought to add that these very simple and very practical manipulations, that are apt at first to discourage diatomists who are little accustomed to laboratory experiment in bacteriology, offer many advantages, especially as in seeking to separate one species you insulate at the same time many others, which you may cultivate at leisure if their study does not interest you at the present moment. Thus, in renewing every six months the pure cultures in new macerations, the diatoms will perpetuate themselves indefinitely in a state of purity, and the fractional separation need not be renewed for the same species during the life of the observer.

I give below three examples of the separation of diatoms with the results obtained, each experiment being taken with twelve sterilised macerations :—

		<i>1st exper.</i>	<i>2nd exper.</i>	<i>3rd exper.</i>
1st macer-				
ation -		Nothing.	Nothing.	<i>Synedra ulva.</i>
2nd „ -		Nothing.	<i>Cyclotella compta.</i>	Nothing.
3rd „ -	<i>Achnanthes exilis.</i>		Nothing.	<i>Synedra</i> and <i>Asterionella formosa.</i>
4th „ -	Nothing.		<i>Cyclotella</i> and <i>Nitzschia palea.</i>	<i>Synedra</i> and <i>Navicula.</i>
5th „ -	<i>Nitzschia linearis.</i>		Nothing.	Nothing.
6th „ -	Nothing.		Nothing.	Nothing.
7th „ -	Nothing.		Nothing.	<i>Synedra</i> and <i>Navicula.</i>
8th „ -	<i>Achnanthes exilis.</i>		<i>Nitzschia palea.</i>	<i>Synedra</i> and <i>Navicula.</i>

	<i>1st exper.</i>	<i>2nd exper.</i>	<i>3rd exper.</i>
9th maceration -	Nothing.	Nothing.	Nothing.
10th „ -	Nothing.	<i>Nitzschia palea</i> .	<i>Navicula</i> .
11th „ -	Nothing.	Nothing.	<i>Synedra</i> and <i>Navicula</i> .
12th „ - <i>Achnanthes exilis</i> .	Nothing.	Nothing.	Nothing.

The first series has resulted in the isolation of two species—*Achnanthes exilis* and *Nitzschia linearis*. The second, of *Cyclotella compta* and *Nitzschia palea*. The third—although actuated by an insufficient dilution, although more than fifty out of one hundred macerations employed, and had been fecundated with one or two diatoms—has yet succeeded in securing in a state of purity *Synedra ulva*, and a little oval fresh-water *Navicula* which I have not yet determined.

You may substitute for the dilution another method that gives good results. It consists in placing a small number of diatoms in a glass containing water, with a layer of gelatinous silica at the bottom. The diatoms reach the bottom of the vessel and fix themselves on the gelatinous silica, whence they may be drawn by glass tubes shaped in the lamp to a more or less capillary aperture, and thus transferred to a nutritive maceration. This is only a variation from the former process.

I have also attempted the separation of diatoms by means of threads (?) placed on fresh gelatinous silica, after the method that Koch employs, to separate the bacteria; but if by that method you may obtain some *Nitzschias*, *Gomphonemas*, or *Fragellarias*, this *modus faciendi* is far from being general, the greater part of the diatoms not being able to grow upon the silica or in the interior of that substance. Possibly, by resuming these studies, and in consecrating to them a great amount of leisure time, we may come to be able to utilise gelatinised minerals for the separation of the algæ that we are studying. However that may be, the old method of division—*fractionnement*—is that which at present gives the best results.

II.—GROWTH OF DIATOMS IN A STATE OF ABSOLUTE PURITY.

In the foregoing cultures we were not specially concerned about the separation of the green algæ, the fungi, the bacteria, and the

infusorians that live concurrently with the diatoms. It now remains to point out the method of separating these other living organisms. We shall have to avail ourselves of the fractional method in most cases.

If the infusoria are very abundant in the artificial cultures, which is not often the case, they are usually got rid of by the operations that enable you to insulate any special diatom.

If, however, this be not the case, a certain portion of the sediment of the maceration in which the diatoms are living should be introduced into a vessel of *distilled* water and left for five or six days, shaking it from time to time. In this innutritive liquid the infusoria which live specially on bacteria will quickly disappear, while the diatoms will continue to live, though they will hardly multiply.

It is essential—especially when you want to study the development of the diatoms—to get rid entirely of these protozoas, which by their continual movements jostle the diatoms, change their position, drag them about; in a word, render all serious and continuous observation impossible. The Rhizopods, besides—as I have had occasion to say—can digest, and consequently destroy, the diatoms, and exercise in the cultures ravages as considerable as those produced by worms and small molluscs.

It is much less easy to clear out the green algæ when they predominate in the sediment or in the cultures. To attain this result, it is necessary to render the siliceous algæ predominant. That done, you resort to the fractional method.

For assisting the predominance of the Pheophyces, the observer has many means at his disposal—means both chemical and physical.

1.—He will eliminate as far as possible from his macerations the salts of ammonia and potassa.

2.—He will carry on his cultures under a subdued light in a place where the luminous radiations are barely sufficient to admit of the diatoms increasing, and he will also employ artificial light and modify it by ground or yellow glass.

3.—He will add to the maceration a dialysed solution of pure silica in the proportion of from 1/10th to 1/5th of a gramme of calcined silica to a litre. The green algæ, injuriously affected by

this substance, develops very slowly in silicified maceration, whilst the diatoms love silica and do not hesitate to get the better of the Chlorophyces. In default of a solution of silica, the experimenter may do a great deal by adding to his macerations a little finely carded asbestos.

When a partial deposit or yellow patches of Diatoms are perceived in cultures conducted after this manner, a small quantity is taken in a pipette, to start a new maceration. It is rare that among these sowings the green algæ are able to maintain their position above the diatoms. When the predominance of these latter is secured, you separate them by the method of division previously described.

The elimination of Fungi must be secured as well as that of the green algæ. The mixture of Fungi and Diatoms is thrown into sterilised distilled water; that is to say, water that has been cleared of all hydro-carboniferous matters. The greater part of the fungi will die in this medium, whilst the diatoms and the green fungi will acquire a certain development, especially if to the distilled water—which is known to be free from all organic matters—a few drops per litre of the solutions A and B be added.* The formula of these solutions was given in the first chapter of this essay.

It now remains to isolate the Diatoms from the Bacteria. This is one of the most difficult manipulations in the technique of the diatomist, for you can only get rid of the bacteria by a series of delicate operations conducted in a most careful manner and requiring special training.

From the moment when the experimenter has determined to clear his macerations of bacteria, he must no longer be content to sterilise his mixtures at 70°C . All, without exception, must be heated to 110°C . for at least half-an-hour, or sterilised by filtration through earthenware; and in conclusion the glass vessel should be raised gradually to 180°C . As the macerations cannot support without injury to their nutritive power a temperature of 110°C ., they should be prepared and sterilised by cold and then, carefully protected from the action of the atmosphere, should be placed in the vessels that have been carefully raised to 180°C .

* See page 37.

You prepare in advance fifty or sixty vessels, half full of nutritive liquid, perfectly sterilised, and you adopt, in order to facilitate the operation, flasks of a medium size, capped and tubulated, of the shape recommended by my friend Freudenreich.

As a rule, the bacteria that are found in cultures of diatoms, from which the green algæ, the fungi, and the protozoa have been

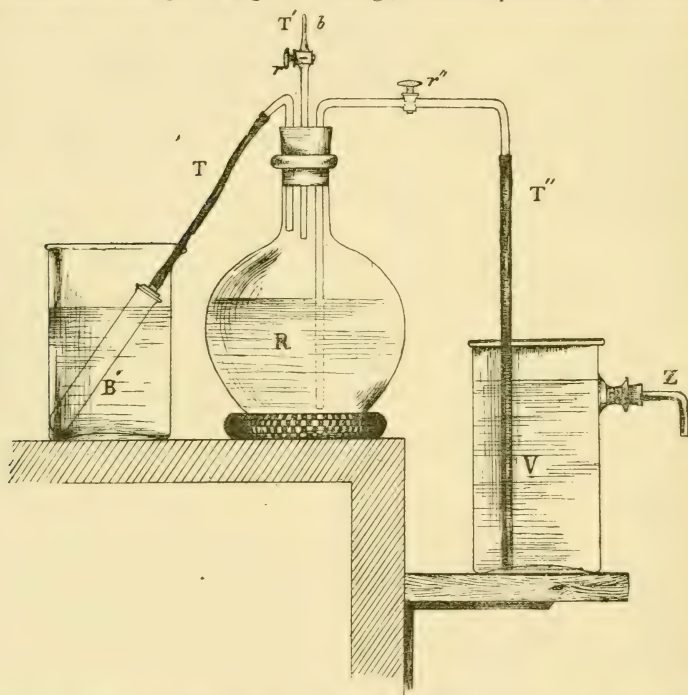


Fig. 64.—Apparatus for separating Diatoms from Bacteria. *R*, the flask; *T*, a tube making the communication between the flask and the atmosphere; *b*, a tuft of wadding; *r*, stop-cock; *T*, a tube conducting the water that has been sterilised by filtration; *T''*, a siphon with a stopcock, *r''*; and *V*, a receiving-vessel, with an outflow, *Z*, to maintain a constant level.

eliminated, are in far greater number than the diatoms. The operation of separation is not practicable directly, and you can only make use of it after you have got rid of the greater part of the bacteria. These infinitely small creatures, being more capable of resisting the action of heat and all other destructive agents, the

operator has only at his disposal the plan of mechanical separation.

To effect this, a convenient quantity of a diatomaceous culture is introduced into a flask, R , previously heated, provided with a sterilised cork, pierced with three holes, in which are fixed three tubes.

The first tube, T , which passes a little way into the neck of the flask, communicating, by means of a tube of caoutchouc, with an earthenware tube, B . The second tube, T'' , goes to within a few centimeters of the bottom of the flask, R ; it carries a stopcock, r'' , and communicates by a tube of caoutchouc, T'' , with a vessel, V , containing water. This tube forms a siphon intended to draw off the liquid from the flask, R .

The third tube, T' , has a stopcock, provided with a tuft of filtering wadding (b), and serves to empty and refill the flask. The maceration liquid being introduced into the sterilised flask, and the stopcock, r' , being shut and a vacuum made, the sterilised water comes through the earthenware tube and fills the flask. You suspend the exhaustion, re-establish the pressure of the atmosphere, open the stopcock of the siphon (which you prime by a little pressure); shut this stopcock and shake the liquid in the flask vigorously. After half-an-hour's rest you decant the liquid by opening the stopcock r , and also r'' , which puts the interior of the apparatus in communication with the atmosphere.

By this first operation you draw off a great proportion of the bacteria. You do it a second, a third, a fourth, a fifth, and perhaps a sixth time; finally, the frustules of the diatoms are by this process rendered more numerous than the bacteria, and only till then do you proceed by *fractionnement*, with the precision needful for bacteriological laboratories.

In summer, if the temperature is high, the flask should be placed in ice, and the water surrounding the earthen tube should be distilled. Out of from fifteen to twenty macerations, fecundated by the *fractionnement* of the liquid from the flask, some will be found that contain no bacteria, which will be shown by the development of the diatoms in a maceration or in nutritive gelatine.

The diatoms, once separated in a state of absolute purity, may be kept indefinitely by employing the well-known precautions

needed in the culture of microbes, already isolated in a state of purity.

Six months of almost daily labour will be required to secure a natural stock of one diatom, even of a common species, *in a state of absolute purity*. I would not, therefore, advise observers to commit themselves to researches, which happily are not indispensable to the study of the morphology of the Diatoms. The cultivation of species in a state of purity, freed from infusoria, green algæ, and fungi, are certainly sufficient. In the meantime, if you wish to study the phenomena of the nutrition of the diatoms, learn what are the aliments that they absorb, the products that they secrete, the changes that they introduce in the mixtures charged with mineral salts and organic matters, the separation of the frustules in a state of absolute purity, is absolutely called for. It is on this account that I have believed I ought briefly to indicate the process—long, it is true, but more complicated in appearance than in reality, which admits of success in this direction.

I do not despair, in the long run, of simplifying this latter method. We shall assuredly come to it when we know better the half-solid mediums which in an especial manner favour the development of the interesting algæ that have claimed our attention.

TEST FOR SESAME OIL.—G. Ambühl states that, though the green colouration of this oil when mixed with nitric and sulphuric acids allows the pure oil to be distinguished from olive, cotton-seed, or earth-nut oil, the detection of an admixture of sesame oil is best effected by shaking the suspected oil with sugar and hydrochloric acid. When sesame oil is present, the watery liquid immediately becomes intensely red. The other oils do not give this reaction, except in the case of the oil from Bari, and that differs from oil mixed with sesame oil in giving the red colouration only after some length of time.—*Schweitz. Wochensch. Pharm.*

Saccharomycetes: A Sketch of the Modern Methods of Classification.

BY H. C. A. VINE.



THE fascinating study of the microscopic organisms, which afford the nearest approach to the physiological unit of life, has, during the last half century, engaged the attention of many master minds both in England and on the Continent. Whether we look at this study in its aspect in relation to disease, to economic value as regards our food supplies, or to the great industries which depend upon fermentation processes as their *raison d'être*, we are at once struck by the vast importance of the issues with which it deals, and we become aware how great a debt humanity and science alike owe to the men who have industriously, and through many years of experiment and failure, ascertained the laws by which these organisms are generated, and the means whereby they may be recognised and controlled.

It is probably in reference to the organisms which form the essential part of commercial yeast that these investigations have been most perfectly carried out, and owing partly no doubt to the fact that, in this case, not only the organism but its environment can be controlled as completely in actual manufacturing practice as in the laboratory, and partly to the great commercial interests involved, which have enabled the research to proceed under conditions which attract to it many rising workers, and provides them freely with every assistance which modern science can afford, with the result that, at the present day, the identification of species among these minute organisms is as certain, if not more so, than it is in higher botany. Some short account of the methods by which these wonderful results have been obtained, and by which the minute cells, averaging not more than 2500th of an inch in diameter, and precisely similar one to another to all appearance under the highest power of the microscope, can, in a properly provided laboratory, in a few hours be classed much in the same way as a skilled entomologist would classify the specimens in a box of lepi-

dopterous insects sent him from abroad, will possibly interest the readers of this Journal.

It will be generally known to the scientific reader that alcoholic fermentation can be brought about in suitable liquids by many organisms other than the Saccharomycetes. The veteran investigator—Pasteur—has shown, in a work published in Paris in 1876*, that *aspergillus*, and the variety which he describes as *torula*, are both able to produce alcoholic change in saccharine liquids. Rees, long since, investigated in Germany the conditions under which *mucor racemosus* becomes an alcoholic ferment, and Brefeld has done the same service in respect of others of the *mucorini*, thus placing it beyond question that the power of producing alcoholic fermentation cannot be looked upon as belonging to any one class, or as in any degree constituting a basis of classification. Indeed, recent researches of Pasteur seem to show that it is not improbably a property inherent in the vegetable cell, and capable of being brought into action in the case of some phanerogamic plants by a mere change in their environment.

It became, then, necessary to seek for a fresh starting point whence to approach the classification of these difficult organisms, and Rees, again taking up the work, and bearing in mind the modern scientific axiom that no classification can be considered of value until the entire life-history of the individual, from one generative act to another, is accurately known, determined to work out the entire cycle of the organisms in commercial yeast, and thus clear up the question once and for ever. He, however, started with the expectation of verifying the old pleomorphic theory which would have made the Saccharomycetes a stage in the life-history of some hyphal fungus, and whilst groping about in the uncertainty in which this presumption had enveloped the subject, alighted on the truth.

His method of investigation was to cultivate a few cells, taken at random from a mass of yeast, upon well cleaned thin slices of potato or carrot, which were placed in a damp chamber, with

* *Etudes sur la Bière*, Cap. IV. :—" . . . Si l'on vient à le submerger de façon que l'oxygène de l'air arrive péniblement en contact avec ses diverses parties il décompose le sucre à la manière de la levûre de bière en formant du gaz acide carbonique et de l'alcool."

certain precautions against accidental contamination from air-borne spores. The neglect of such precautions in former experiments of like nature had led to the invasion of various moulds, and had given rise to the now exploded pleomorphic theory. The cells thus placed under suitable conditions for observation, were maintained at a medium atmospheric temperature for some days. At first, some development by the ordinary process of sprouting took place, so that the circumference of the patch of yeast extended itself beyond the space originally occupied; but the new cells produced had few or no vacuoles, and, at the end of three or four days, this method of increase entirely ceased, and the character of all underwent a change. Some cells appeared empty and died out, in others the protoplasmic cell contents assumed a granular appearance, and in a few hours showed a tendency to concentration on independent points within the cell. This increased until several distinct protoplasmic masses were formed, which eventually became covered with their spherical envelopes while still remaining within the mother cell, the wall of which had distended and, at the same time, gained in tenuity.

When this development appeared to be fully matured, these compound cells were placed in suitable nutritive liquids, and in a short time new cells were produced, not from the mother cell, but by the budding of the newly formed spores, these buds penetrating through the old cell wall, which speedily disappeared. The cycle of changes was thus found to be complete, and the constancy of the organism under the altered conditions of reproduction (which had hitherto been fiercely disputed) was fully established by the demonstration of a stage in its history which proved its proper position to be among the ascomycetes, and which fungologists at once recognise as a form of spore formation in an *ascus*, the mother cell itself constituting the *ascus*.

The next step of importance undertaken was to ascertain how far this process of *ascospore* formation is general among the organisms known to cause alcoholic fermentation, and here the work was taken up by the able hands of Dr. Chr. Hansen, of the Carlsberg Laboratory, at Copenhagen. Seeking for a character which should enable him to define the genus *Saccharomyces*, this skilful observer made an exhaustive investigation, and found that those

species which ordinarily constitute commercial yeast, and to which the name of *Saccharomyces* was originally applied, are alone in reproducing themselves by spores formed in this particular way. This fact, being fully confirmed, on further investigation, by Hansen himself and others, constitutes a definite test, which enables the genus to be defined strictly as *an alcoholic ferment reproducing itself both by sprouting and by means of ascospores*. From this stage in the investigation Dr. Hansen was enabled to build up, with wonderful patience and skill, by means of investigations lasting over many years, the system of classification now employed.

As far back as 1878 the writer, being then engaged in technical research, became convinced that the apparently similar cells in yeast were by no means identical; in fact, that some fundamental difference existed between different samples, and probably between the cells constituting each. The proof of this was found in the fact that whatever care might be exercised in the microscopic selection of yeast, the result of the action of different samples, in the same nutritive medium, exhibited wide analytical and practical differences. That is to say, when two different yeasts, between which no difference could be observed on most thorough microscopic examination, were cultivated in sterilised malt wort under precisely similar conditions, the resulting alcoholic fluids frequently yielded altogether different analytical results. Want of time, and the necessity of carrying on daily technical work, prevented the following up of research thus suggested, but soon after Hansen, who was then engaged on the subject, took it up at this point, and starting from the ascospore formation discovered by Rees, he applied it with unlooked for success as a means of identifying and separating the varieties of cells in commercial yeast.

It was, at an early point, evident that to obtain results of any value he must work with *pure cultures*; that is to say, yeasts which had been grown under such stringent conditions as should insure the presence of *one* species only. Such a culture could only be obtained by the selection of a *single cell* and allowing it to multiply, with proper precautions against accidental contamination, until a sufficient bulk was obtained for experimental purposes. The great difficulty to be overcome was the introduction of a single cell, and *no more*, into the flask in which the

culture was to take place. Hansen adopted the following method, which is now in general use :—By the addition of a small quantity of yeast to a sterilised malt infusion contained in a Pasteur flask, a vigorous fermentation is started. When the process is well advanced the liquid is poured off, and the yeast which is adherent to the bottom of the flask is diluted with sterilised water to what seems likely to be a suitable extent. This mixture is well shaken, and from it a small drop is removed upon a glass point or fine platinum wire. This drop is placed upon a slide, and at once covered with a cover-glass ruled into squares, such as is used for counting blood-discs, by the aid of which the cells present are counted under the microscope. It is important that the drop of liquid employed be barely sufficient to extend under the whole of the cover, as if any escapes beyond the margin the experiment is valueless. Let it be supposed that fifteen cells only can be observed in the drop. Then the flask containing the diluted yeast is again shaken, so as to thoroughly diffuse the cells through the liquid, and a similar drop to that counted is transferred to a flask containing 30 c.c. sterilised water. There is then a probability that this flask contains about fifteen cells. A number of flasks containing sterilised malt infusion being provided, the flask containing the drop of diluted yeast is thoroughly shaken, and 1 c.c. of its contents quickly transferred to each of the infusion-flasks, which should number at least twenty. It is pretty certain that a number of them will have received one single cell and no more, but it is of the greatest importance to ascertain the fact by actual evidence. For this purpose the flasks are at once subjected to a prolonged and vigorous shaking, either by hand or by mechanical means, so that if two or more cells have found their way into one flask they may be separated one from the other. The flasks are then set aside for some days at a suitable temperature, when they are carefully lifted and examined. If only *one* white speck has formed on the bottom or sides, it is clear that only one cell has found its way into the flask and a pure culture has been initiated. Those flasks which exhibit more than one spot of vegetation or in which no growth is visible, are rejected as useless.

When, on these lines, Hansen had obtained his uncontaminated cultures from single cells, he was in a position to compare

their behaviour under various conditions, and in particular as to the mode in which they formed ascospores. Evidence was soon apparent that the conjectures which had been made as to the variety of species in this country and abroad were entirely correct, and that the cultures of *Saccharomycetes* differed considerably one from another, both as to the temperature and time at which spores were formed, and that these variations were of a regular and definite character, and were coincident with well marked changes in the action of the organism on the liquids in which it was cultivated. The skill of the observer enabled him to reduce these facts to a tabulated form with such accuracy that the investigation yielded not merely a means by which the limits of the genus might be ascertained and defined, but a method by which the varieties within the genus might be differentiated on a basis of classification as exact as that afforded by the reproductive organs of the higher *Cryptogamia*.

In the course of time the details of working have been considerably modified, the use of slices of potato or carrot as a cultivation material having been early abandoned in favour of tablets of porous earthenware, of gypsum, and latterly of gelatine, which last is especially favoured as affording a very convenient means of direct microscopic investigation of the growing cells from time to time.

Several years were employed in these investigations. Other methods—notably, the characteristic indications yielded by the “mother,” or “*voile*,” as the continental term is, which is formed under suitable conditions by the different species upon the surface of the liquid in which they develop—were employed to confirm the results obtained. The writer has had opportunity to work out these problems as regards three varieties—those described by Hansen as *S. Cerevisie* I., and *S. Pastorianus* II. and III.—with the following results, which are sufficiently near to those obtained on the Continent to be considered satisfactory. The production of spores in these three varieties ceases entirely at a temperature above 98° or 99° F., and becomes very slow below 55° F., ceasing altogether at 35° F.

The periods of spore development at 62° F. (which is a convenient temperature for general working) varies from fifty hours in

S. Cerevisiæ I. to thirty-six hours in *S. Pastorianus* II. The diagram which accompanies this article embodies these results in a way which admits of ready comparison, the differences between the species being so marked when the cultures are made at a temperature of 52° F. as to indicate pretty correctly to which species each belongs. The experiments recorded in this diagram were made with a laboratory yeast, which, no doubt, had a Continental origin, and the figures thus obtained do not hold good for English "high-fermentation" yeasts, which have been cultivated under very different conditions of temperature and nutriment to those employed above.

The details of the experiments by which the results shown on the diagram were obtained will be of interest to the reader. Fully-developed but somewhat inert cells, of a pure culture in each case, were transferred to suitable flasks of sterilised malt infusion, and allowed to develop for twenty-four hours, when the liquid was carefully poured off and a fresh supply of the same malt infusion introduced. This is necessary in order to obtain thoroughly vigorous cells, from which alone spores are likely to be formed. At the end of another twenty-four hours—(the time must be observed precisely)—the deposited yeast was removed by means of sterilised platinum foil or wire to gypsum and gelatin tablets (of course, sterilised), which were placed in suitable appliances for maintaining constant temperatures. A careful and frequent microscopic examination was then kept up in order to detect the earliest traces of spore formation, the time of the first indication of such change being carefully recorded against the temperature at which the culture had been maintained. Similar observations were simultaneously made on a series of cultures from the same yeast, carried on at various temperatures, and the results showed, as was expected, a regularly-varying period of spore development for each variation of heat, which, being properly registered, may be made the basis for a chart somewhat similar to that employed in recording changes of temperature in the human subject. The chart attached to this article contains the curves for the three species named, which have been the subject of special experiment.

It will be readily understood how the data which can thus be obtained without much difficulty in a physical laboratory,

where the means of research are at hand, afford an easy mode of identifying the species of any particular culture by a comparison of the figures obtained with those indicated by the curves registered on the standard laboratory charts. By a further development of the process, the varieties present in a mixed yeast may be separated and determined—always provided that they are amongst those whose temperatures and periods have been ascertained experimentally.

The technical importance of this knowledge to the trades which depend upon fermentation as their chief process can scarcely be over-rated, though it has borne less fruit in this country than on the Continent, owing to the different lines on which fermentation processes are carried on. Its value hinges on the fact that each variety produces special characters in the liquid in which it has developed, and in which it has converted a greater or less part of the hydro-carbon present into alcohol. Thus, *S. Pastorianus* II. (Hansen) produces an intense acrid bitterness in the beer in which it multiplies which is especially dreaded by the German brewers. It is said that the accidental presence of this flavour in the beer of the Carlsberg Brewery in Denmark, and the ability of Dr. Hansen to prove to the proprietor, Capt. Jacobsen—a name well known in many scientific circles—that it was due to the presence of this objectionable ferment that first led to the practical introduction of pure cultures of yeast—or, rather, of *suitable* varieties of *Saccharomyces*—into industrial enterprise on the Continent.

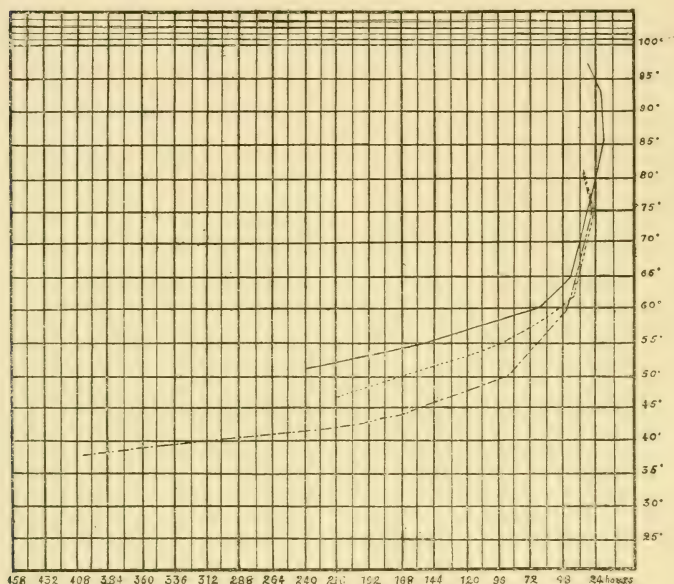
The result of this new departure fully justified the confidence with which the technologists had urged the attempt. The flavours imparted to the saccharine liquids in which the fermentations were carried on were completely controlled by the variety of yeast-cells furnished from the laboratory, and so perfect has the knowledge of working in this respect now become that pure cultures of various species, mixed in such proportions as to suit the requirements of any particular brewery, are now regularly produced in Continental laboratories in quantity sufficient for ordinary working purposes, each variety having been originally grown from a *single cell*. Several leading manufacturers have made patient efforts to utilise the application in the brewing

industries in this country; but so far, it is understood, without much success, owing, as it is generally believed, to the difference of the working conditions obtaining in English breweries, where the fermentation, conducted in a strong malt infusion at temperatures ranging from 55° to 80°F., differs greatly from the slow process carried on at 38° to 50° F. in a malt wort of low specific gravity, which is general in Germany. The writer has good reason to think, from his own observation, that not only the conditions of growth differ, but that there is a distinct *racial* difference, so to say, between English (*fermentation à haute*) and Continental (*fermentation à bas*) yeasts. The indications of this are especially marked among the yeasts employed in old-fashioned Scotch breweries, where for generations "changes" have been avoided, but where absolutely necessary they have been obtained from a local brewery of the same class, with the object of *preserving the peculiar characteristics* for which Scotch beers are known. This would, of course, tend to preserve the type of yeast unchanged, and accordingly it is among those that the widest and most marked divergencies from the Continental type are to be found, the interval being to some extent filled up by the yeasts in use in different districts in England. The limits of the present article do not admit of entering upon this attractive field, about which more may be said on another occasion. The method of identifying species by the separation of a single cell and its culture under fixed conditions of temperature, until the formation of spores is observed to commence, has been explained, and it is hoped that with the aid of the chart on the next page the reader will have no difficulty in obtaining a clear comprehension of this most interesting subject.

On the reopening of an old mine at Bangor, Cal., U.S.A., a few months ago, flies were found in a dry slope connecting two shafts, all white except the eyes, which were red, and a white rattlesnake was killed. The animals had lived in the dry passages, where they had been supplied with air, but not with light. A few of the flies, exposed to light in a glass case, recovered their proper colour in a week.

DIAGRAM

Showing the Development of Ascospores in three species of Saccharomyces under varying conditions of time and temperature.



SACCH. CEREVISIÆ, I.

100° no change.

98° indications of spores at 29 hrs.

95° " " 26 "

93° " " 22 "

86° " " 20 "

75° " " 25 "

65° " " 45 "

60° " " 66 "

55° " " 150 "

52° " " 240 "

48° no spores form.

SACCH. PASTORIANUS II.

84° no change.

82° indications of spores at 34 hrs.

76° " " 26 "

65° " " 38 "

59° " " 48 "

50° " " 90 "

46° " " 160 "

38° " " 400 "

35° no spores form.

SACCH. PASTORIANUS III.

82° no change.

81.5° indications of spores at 34 hrs.

76° " " 28 "

71° " " 30 "

65° " " 40 "

61° indications of spores at 45 hrs.

55° " " 95 "

50° " " 168 "

47° " " 216 "

44° no spores form.

The Human Skin : Its Structure and Functions.*

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HAVE chosen this subject for my lecture to-night for several reasons; one is, that it can be considered more quickly as a whole than many physiological subjects, though, in the brief space at my disposal, it is impossible to give more than the barest outline; another reason is its importance to health; a third is that there are so many erroneous ideas about its structure and functions. I feel that in dealing with this subject I must commence by referring to a few physiological axioms, as it will save much explanation afterwards. You are all acquainted with the meaning of the term "Cell," as connected with living things. Briefly, the definition of a cell is a "Microscopic portion of living matter or protoplasm having a distinct life history of its own." It is, in fact, a little mass of living material which is practically, as regards its life history, distinct from everything around it. Of such a typical cell we have an example in that low form of water-life, the Amœba. This creature is unicellular; that is, it consists of one single cell, just as every human being once consisted of a single cell, the Ovum. This minute Amœba, which is more like a speck of jelly than anything else, is yet an individual being, and goes through its life-history as essentially separate from other Amœba as you or I go through ours. From the moment that it is born from a parent Amœba it has to obtain, eat, and digest its own food, grow to maturity, move about, reproduce its species, decay, and die as certainly as any human being, and, as a rule, with far more regularity of habits.

If, however, we ascend the scale of living things, we find that the members of the series become more elaborate in their structure.

* A Lecture delivered before the Sheffield Micro. Society, Feb. 10, 1893.

Instead of consisting of one single cell they consist of several cells joined together into a little colony, more complexly arranged as we get higher in the scale. They become, in fact, multicellular, or many-celled organisms. I can best compare the difference in the two conditions by a familiar simile.

Imagine for a moment a small primitive country village, such as we still happily find in Derbyshire, in which a small tradesman keeps the little shop, for it is usually the only one, where he sells everything—meat occasionally, boots, groceries, peg-tops, hats, stockings, black-lead, and postage stamps. He is his own salesman, takes the money himself, buys in his own stores in the neighbouring town, puts up his own shutters, and sweeps out his own shop. He does, in fact, everything himself, but it is, necessarily, in a very small way. There you have a unicellular structure, a very primitive arrangement, such as the lowly *Amœba*, perfectly fulfilling all the village requirements. Let us imagine, however, that years afterwards, owing to some cause or other, such as the discovery of coal in the district where our village stood, it has grown up into a large and populous town: railways, telegraphs, and telephones, the later discoveries of science, have made the little village shop quite inadequate for the demands of the inhabitants, and, on its former site, there stands an immense Great Northern Supply Stores, with hundreds of assistants employed in its work. One group of them now looks after the meat department, another the boot department, and so on; whilst a special set of cashiers takes the money, and others have as their duty to sweep out the Stores, and put up the shutters; the whole of them being under the governance of the Directors of the Company. There you have a picture of a multicellular shop; a complex organism, such as the human body, where the myriads of little cells are divided into groups, each of which groups has some special functions to perform with relation to the harmonious working of the whole, and all of which are under the direct control and supervision of the Directors, *viz.*, in this case, the Central Nervous system.

In the human body, then, the cells can be broadly divided into groups, each of which has its own particular structure and function, or functions, and each of these groups is called a Tissue. Thus

there is Muscular Tissue, Nervous Tissue, Connective Tissue, and others, all working together for the common good of the organism. The advantages of this division of labour and co-operation is very great, and quite self-evident, as it enables things to be accomplished which, in a unicellular organism, could not be attempted.

It is only with two of these departments or tissues, or, rather, with part of two, whose work is combined and blended together, that I propose to deal to-night:—1st, The Epithelial Tissue, or Epithelium; and 2nd, the Connective Tissue, the layers of which form the skin. Of course, we must bear in mind that in discussing any individual department of a large establishment, we cannot isolate it entirely from the rest, but shall have to frequently consider its relation to other departments, such as, in this case, the Blood, Muscular Tissue, and especially the Central Nervous System.

The skin consists of a mass of cells arranged in two distinct layers:—*A*, More superficial—Epidermis; *B*, Deeper—Cutis Vera, Dermis, or Corium. The former is epithelial, the latter is connective-tissue, which, in various conditions, *e.g.*, fibrous, cartilaginous, and bony, forms the framework of the body.

The upper or epithelial portion consists of several superimposed layers of cells lying close together, with intercellular substance joining them, and arranged much like bricks in a wall. The cells vary in shape from the lower layers upwards; thus, the deepest layers of all are somewhat cubical in shape, whilst as the higher layers are reached they become more and more flattened and scale-like. The lowest layer is very uneven, as it has to fit over the dermis or true skin underneath, which, as we shall see, is constantly rising into little projections or papillæ.

The cells of the different layers or strata also differ in their chemical composition: the deepest stratum consists of cells whose composition approaches that of ordinary living protoplasm, whereas the superficial strata become horny in character, so much so that, in parts where there is much pressure, such as the palms of the hands and soles of the feet, this horny layer is much developed; a condition well seen in the hands of those occupied in various Sheffield trades, *e.g.*, those who do much filing, and many others.

This difference in chemical composition causes also a difference

in the appearance of the strata of epidermis on vertical section, so much so that the various layers have been described separately, and are usually divided into four :—

- 1.—Stratum Malpighi. 2.—Stratum Granulosum.
- 3.—Stratum Lucidum. 4.—Stratum Corneum.

The constant friction of the body surface against external surroundings causes the superficial layers to be constantly rubbed off, and these are replaced by the next row below becoming the superficial layer, and in turn being rubbed off ; so that, in a short time, the epidermis would be rubbed away altogether were it not that new cells are constantly being formed in the lower portions of the Stratum Malpighi, and push upwards the upper layers. The epidermis contains no blood-vessels.

We now come to the lower, or true skin—the Dermis. You are all aware, by painful experience, that the epidermis can be removed during life, and expose below the true skin ; if not, the experiment is a simple one :—Knock your knuckle against the catch of the door or corner of the table, you will then see the exposed dermis, in which the blood vessels are running, looking red and bleeding slowly. It is exceedingly tender ; place the injured finger in soapy water and prove this for yourselves ; for it contains the nerve endings, or communications with the central nervous system, which is, by their stimulation, informed of something wrong in the skin department ; just as surely as the breakage of crockery in the China department of our hypothetical Supply Stores will reach the ears of the Directors, causing them pain and annoyance, which will be the greater, the greater the damage.

If we examine this dermis in vertical section under the microscope, we find that it is a much looser material than the epidermis, consisting of interlacing bundles of wavy fibres running in various directions, with a few cells scattered here and there ; the whole being something like a dense layer of straw. Running all through this layer is a fine network of blood vessels, of very small size, and the fulness or emptiness of these gives the redness or pallor to the skin. Moreover, the whole thickness of the dermis is soaked or saturated with a clear yellowish fluid, which has exuded from the walls of the blood vessels, and is called lymph. This lymph becomes very evident, in the little experiment of knocking the

knuckle, after it has stopped bleeding, for you must all have noticed how a pale yellow clear liquid oozes then from the sore place, and soon forms a scab or protection to the wound, enabling the process of repair to be carried on. In the dermis, also, we must not forget the nerves, with their curious nerve-endings, very varied in form, and constituting the sense-organs of touch. They are frequently small oval bodies, like little seeds, situated on the nerve fibres.

As we proceed to the deeper parts of the dermis, the fibrous tissue becomes less dense, and, at a certain depth below the surface, we find little dark bodies—very well shown in the sections—the sweat-glands. As these have such important functions to perform, I must ask your permission to dwell on them briefly.

Let us ask, in the first place, what is a gland? It is an organ which has for its functions the manufacture of some fluid which has a definite composition and definite action. In its simplest, or its most elaborate form, it is nothing more than a single row of epithelial cells, lying on a basement membrane, with blood-vessels underneath, the cells being free to the surface at their opposite side.



FIG. 65—Simple secreting surface, or Mucous Membrane. *A*, Free surface; *B*, Epithelial Cells; *C*, Basement Membrane; *D*, Fibrous coat; *E, F*, Blood-vessels.

These epithelial cells pick out material they want from the blood circulating below them. They eat these, and, in the eating of them, manufacture certain new materials, which they pour out on to the open surface in the form of a liquid, called the Secretion of the particular gland in question, *e.g.*, the Saliva, Gastric Juice, Bile, Sweat, etc. Thus, the sweat is the secretion or product of the activity of the epithelial cells of the skin; but in our highly elaborated bodies instead of all the epithelial cells having this function, it has been especially relegated to certain portions of them, and they, instead of lying flat on the surface, dip down into the skin, and there coil about so as to increase many times the

As we continue to look at the still deeper layers of the skin, we find fat-cells and muscular tissue in abundance. The contraction of the latter is often demonstrated to us, in every-day life, by the condition known as goose-skin, when the surface of the body becomes chilled.

Below this fatty layer the skin becomes much less dense, the fibres are further apart, and fat is sometimes enormously deposited. This is called the subcutaneous layer. It is at this point that the skin separates from the muscles below, as in skinning an animal.

Time does not permit me to speak of various modifications of the skin, such as the hair and nails. Neither can I discuss the curious furrows and ridges on the fingers, which have so absolutely different an arrangement in different persons, and yet are so permanent all through life, that Mr. Francis Galton, F.R.S., has suggested a system for recognising old offenders in crime by keeping the wax impression of every criminal's finger-prints for reference in future offences.

But I must turn for the brief remainder of my lecture to the Physiological functions of the skin, which, I hope, may prove of even greater interest than its structure.

We may divide them under six heads :—

1.—Protective. 2.—To prevent the rapid loss of heat or absorption of poisonous materials. 3.—To regulate the body temperature. 4.—To secrete sweat. 5.—The special organ of touch. 6.—Absorption.

The first function is self-evident and need not detain us.

The second is of more importance, especially to those who have medical work to perform. Thus, we know that in severe burns or scalds the gravity of the injury depends more on the extent of surface over which the skin is destroyed than upon the depth of the burn.

The third function is of especial importance to all of us. The human body in health maintains a constant temperature in spite of the changes around it. Thus, if we journey to the frigid Iceland or to tropical Africa ; if we drive over the moors in a biting nor'-wester, or recline on a slab in the hot-room of a Turkish bath, a thermometer placed in the mouth would register about 98·6° F. This temperature may be increased or diminished in disease, but

only within comparatively narrow limits. Thus, in what we call fever, the body-temperature may go up to 105°F. or 106°F. ; but this itself is not altogether without risk, especially if continued for long, and a temperature above this is mostly fatal. Again, loss of heat below a certain point will itself prove fatal; hence the value of warmth in restoring life after immersion or exposure to severe cold of any kind.

How this constant temperature is maintained is full of interest.

It is evident that in a cold climate we must manage somehow to lose less heat, and in a warm climate lose more in order to keep our temperature equable. This regulation of expenditure of heat is chiefly the duty of the skin, governed by the central nervous system, and is performed in three ways:—First, by conduction; second, by radiation; third, by evaporation.

By conduction we mean the loss or gain of heat by contact with colder or warmer bodies. Thus, putting an ice-bag on the head will cool it, and the application of a warm bottle to the feet will warm them. We can in this way vary somewhat our expenditure of heat by placing ourselves in contact with hotter or colder bodies, and chiefly by putting on clothing composed of materials which are bad conductors of heat, such as wool, flannel, etc.

Secondly, by radiation. By this we mean the heat given out in rays by a glowing body, such as the sun or a fire, which falls on and heats objects placed in its rays, but does not raise the temperature of the intervening medium through which it passes. This is thoroughly understood in the practical form by young ladies, who know that radiant heat does not improve the complexion, and therefore use parasols, fire-screens, etc.

Just like any other heat-generating bodies, we give out more or less radiant heat, accordingly as the blood-vessels of the skin are distended with blood or contracted and empty. Stand by a man who has just finished a football match or run a mile race. His skin is red and glowing, the blood-vessels are full, and you cannot fail to perceive how much radiant heat he is giving out.

Similarly, observe your fellow-men in a Turkish bath. They look like boiled lobsters, for the hot blood has, to borrow an electrical term, been switched off from the internal parts of the body and is coursing chiefly through the blood-vessels of the skin,

because the loss of heat has to be increased in the hot surroundings of a Turkish bath.

Take the reverse picture : a cold winter's day. Most of us look pale and pinched. The blood-vessels of the skin are empty and the skin itself feels cold. The amount of radiant heat given off is diminished, for the blood has been switched back to the blood-vessels of the internal organs.

Artificially also we regulate our loss by radiation, by exposing or covering the surface of the body.

Thirdly, we can lose heat by evaporation of moisture from the skin. The sweat-glands are constantly pouring out small quantities of secretion at the orifices of their ducts ; but this is not apparent to us, because as quickly as it is formed it is evaporated or converted into vapour by the heat of the body. If, however, the surrounding conditions of atmosphere, etc., require an increase in the loss of heat, the secretion of sweat is much increased, and the fluid so poured out cannot be converted into vapour sufficiently quickly ; it therefore lies on the skin as drops or beads of sweat. This is popularly called sweating ; but please remember that, physiologically speaking, we are always sweating, and it is only when either the excess of secretion, or the prevention of the ordinary amount of evaporation by a moist condition of the atmosphere on a calm, muggy day hinders its conversion into vapour that the sweat becomes visible, or, as it is called, "sensible sweat." Protected by such a perfect regulating mechanism, it has been possible for experimenters to enter rooms the temperature of which seems fearful, and would be rapidly fatal were it not that the air in them had been absolutely dried, so that loss of heat by evaporation could be fully carried out.

Thus more than 100 years ago two observers were able to remain with impunity in a dry chamber heated to 260° F., and with ease in one so hot that it became painful for them to touch the metal buttons of their clothes. And, as you all know well, a bath at 120° F. is, to say the least, uncomfortable, whereas that temperature in the hot-room of a Turkish bath is nothing.

Other instances of this regulation of the temperature by loss of heat readily explain themselves, and it is needless for me to say more.

The 4th function I will not say more about, and the tempting subject of the 5th function—namely, the Special Sense of Touch—I dare not begin. Suffice it to say that this sense is far more acute in some parts of the body than others. Thus, if you take two blunt needles, and try how near together at various parts of the body you can feel them as distinct points, you will find that at the tip of the tongue and on the finger-ends sensation is most acute, and so on.

And now the last function—that of Absorption. Its extent is exceedingly limited. The great value of ointments is in their local action, and probably their absorption into the vascular system is very slight. When people talk of rubbing a weakly child with cod-liver oil to fatten it, I always advise them to let the child drink the cod-liver oil, and rub it with a good rough towel every morning after its bath, for, as far as regards the absorption of cod-liver oil by the skin, they might as well rub it into the child's macintosh. It is the friction of the skin that is wanted.

In amphibia—frogs, etc.—the skin acts as a respiratory organ, important as the lungs; but in man this function is at the most exceedingly slight, if present at all.

One or two words I would say in conclusion :—

First, the great value of baths, followed by friction of the surface. And under this head I would particularly mention the Turkish bath as a most valuable combination of every hygienic method for the skin. It is much too little used by the public. This is a pity, as it is especially refreshing and invigorating to those whose daily life in a city like ours is often of a sedentary character, in whom the lack of active daily exercise prevents a proper action of the skin. Many so-called bilious attacks are, I am convinced, merely due to defective elimination of waste products, and may be averted by a Turkish bath, which is cheaper and far more pleasant than going to a doctor. Try it for yourselves, and when you do, remember to stay there at least three hours, taking at least one and a-half hours in the cooling-room, lying quietly before going out into the open air, as you will thereby avoid the least danger of catching a chill.

Secondly, as to the morning cold bath, the Englishman's pride, it is an excellent thing for those who can get warm again imme-

diately ; but if not, if you get out feeling cold and shivery, do not continue it, but add a little warm water to make it tepid, or merely sponge with tepid water, and rub yourself well with a dry towel. Its value consists in exercising the cutaneous blood-vessels to contract and relax rapidly, when exposed to sudden changes in the temperature.

Lastly, please remember that feeling a sensation of warmth does not mean that the body temperature is raised, but, in itself, shows that, by the glowing skin, the necessary process of cooling is taking place.

Polarised Light and its Applications to the Microscope.

BY G. H. BRYAN, M.A.

PART III.

Chromatic Polarisation.—We will now examine the cause of the pretty colours displayed by certain polariscopic objects, and more especially by crystals, films of selenite, and rock sections. To exhibit good colours, the object must be *neither too thin nor too thick*. When a rock section has been ground fairly thin, it shows splendid colours ; but on grinding it still thinner and thinner, all the colours disappear, and at last the only effect remaining is that certain portions of the section appear greyish white when the polariscope is adjusted to give a dark background.

In preparing crystals for the polariscope, too, they must not be allowed to form either too thick or too thin a coating on the slide if pretty colours are desired. If too thin, the crystals will appear of a greyish white colour on the dark background ; if very thick indeed, they will again appear white ; but the best colours will be obtained with certain intermediate thicknesses.

Again, the thinner animal hairs only appear white when the polariser and analyser are crossed ; but thicker hairs, such as those of the Polar Bear (which is thus “polar” in two senses of the word), exhibit fine colours.

The Colours of the Spectrum.—To explain these colours by

means of the wave theory, let us for a moment consider what constitutes white light. If a small pencil of sunlight is passed through the prism of a spectroscope, we get a band of different colours, called the *spectrum*, and if a second prism is so placed as to counteract the separating effect of the first, these colours will be re-combined and will again form white light. The order of the colours in the spectrum is:—*Violet, indigo, blue, green, yellow, orange red*. Now, in the wave theory, these different colours represent different rates of vibration of the ether, just as the different notes of the musical scale depend on the rates of vibration of the sound-waves which strike on our ear. The slowest vibrations which produce any impression of light on the retina of the eye belong to the extreme red end of the spectrum, and their frequency is *399 billion* vibrations in a second. The quickest vibrations visible in the form of light belong to the extreme violet end of the spectrum, and of these *831 billion* strike the eye in a second. Thus, the rates of vibration in the extreme violet and red rays are in the proportion of about *two to one*—the same relation that holds between a note and its octave in music; in other words, *our range of vision extends over about an octave*. Roughly speaking, the velocity of light is the same for all colours, and as a consequence the *wave length* or distance from one wave to the next in a ray of red light is about double the wave length in violet light. Fig. 6 shows the proportions of the wave lengths in red, green, and violet light, enormously magnified of course, for when the light is travelling in air there are really about 33,866 such waves of red, 43,197 of soda yellow, and 70,555 of violet light in the space of a single inch.

The colours of the spectrum are pure, but most ordinary colours contain a certain mixture of these pure colours. And from what has just been said, we see that ordinary white light consists of a mixture of all the colours of the spectrum.

Effect of Colour on Polariscopic Appearances.—In the last section we showed that there is a certain periodicity in the changes of type that take place in a beam polarised at an oblique angle with the optic axes of a doubly-refracting section through which it is passing. And these changes were shown to depend on the ordinary and extraordinary rays travelling through the section at

slightly different rates, a complete cycle of changes taking place when the gain of the quicker component ray amounts to a whole vibration. Now, the thickness necessary for this cycle varies for various colours ; consequently, the rays of different colours, after passing through the section, are differently polarised, according to the types of vibration represented in Figs. 5 ($a-k$). These rays behave differently when passed through the analyser, and therefore the section appears coloured. The colours of selenite are not pure, but are formed of a mixture of all those component colours which the analyser transmits.

A few simple numerical examples will make this clear.

First.—Let us take the frequencies of vibration to be 400 billion vibrations a second in red, 600 billion in green, and 800 billion in violet light. Let us suppose the thickness of a film of selenite to be such that the “extraordinary” ray takes *one 800-billionth* of a second less time to pass through it than the ordinary ray. This gain of time in the extraordinary ray amounts to a whole vibration for the violet light or half a vibration for the red light. Consequently, the violet on emerging has exactly undergone its complete cycle of changes, while the red has only undergone half its cycle. The violet comes out polarised as at (k), Fig. 5, and the red as at (e). With the Nicol’s prisms crossed, the red light is all transmitted and the violet all quenched ; with the Nicols parallel, the red is quenched and the violet transmitted.

The green light emerges in an intermediate stage—viz., circularly polarised as at (g). It is partly transmitted whether the Nicols are crossed or parallel. Of other intermediate colours, those near the violet end—viz., blue and indigo—are transmitted with greater intensity with the Nicol’s prisms parallel than with them crossed ; while those near the red end—viz., yellow and orange—are transmitted with greater intensity with the prisms crossed than with them parallel.

The effect on the whole, then, is to give the selenite a blue colour for one position of the prisms and an orange colour for the opposite position. Thus the colours in a film of “blue and yellow” selenite are accounted for.

Second.—Suppose the thickness of the film doubled so that the extraordinary ray in passing through gains *one 400-billionth* of a

second on the ordinary ray. This difference of time amounts to 2 vibrations for violet, $1\frac{1}{2}$ for green, and 1 for red light; consequently, on emerging, the violet has undergone exactly 2 cycles, the green $1\frac{1}{2}$, and the red 1 complete cycle of changes. The violet and red come out polarised as at (*a*) or (*k*), Fig. 5, and the green as at (*e*). With the Nicols' prisms crossed, we get green light transmitted, but no red or violet; with the Nicols parallel, red and violet are transmitted and green is quenched.

As before, the intermediate colours are affected in an intermediate manner. We get on the whole purplish red for one position of the prisms, and green for the opposite position. The the colours in a film of "red and green" selenite are accounted for.

Third.—If the thickness of the selenite is now increased very slightly, the colours in which one ray gains $1\frac{1}{2}$ and 2 vibrations on the other, will correspond to rather slower rates of vibration than before. Instead of green we shall get yellow light with the Nicols' prisms crossed, and instead of violet we shall get blue with the prisms parallel. We here again have a film of blue and yellow selenite, but the tints are not quite the same as they were before.

Fourth.—For a much greater thickness a number of different colours are transmitted when the prisms are crossed, and a number of other colours are transmitted with the prisms parallel. The resulting colours are more mixed than they were before, and are consequently much less conspicuous. When the section is very thick, this mixture gives rise to practically white light in every position of the analyser, though if this white light is examined with a spectroscope it will still be found that a number of colours are missing in either position.

Fifth.—For a very thin polariscopic object we again get white light with a dark background. In *all* the colours the change of type produced by the object is only sufficient to make the emerging light slightly elliptically polarised, the directions of the light-vibrations being changed from straight lines to narrow ellipses, as at Fig. 5 (*b*). The change has most effect on the quicker vibrations, which are therefore most strongly transmitted by the "crossed" analyser; but all colours are more or less transmitted. Hence the object appears of the rather *blueish*-white tint with which we are so familiar.

Colours of Selenite for different thicknesses.—We hence see that the colour of the selenite depends on its thickness, and the above examples show how it would be possible to calculate out from first principles what would be the colour for *any* thickness. The sequence of colours for different thicknesses, beginning with the thinnest, is given in the following table :—

COLOURS OF SELENITE OR NEWTON'S RINGS.

		Colours of Selenite, with prisms crossed, or colours of Newton's Rings for reflected light.		Colours of Selenite, with prisms parallel, or colours of Newton's Rings for transmitted light.
1ST ORDER	...	Black	...	White.
		Pale blue.	...	Yellowish brown.
		Bright white.	...	Reddish violet.
		Pale yellow.	...	Indigo.
		Orange.	...	Blue.
		Red.	...	Green.
2ND ORDER	...	Purple.	...	Yellow.
		Blue.	...	Orange.
		Yellowish green.	...	Reddish violet.
		Yellow.	...	Indigo.
		Crimson.	..	Green.
3RD ORDER	...	Purple.	...	Greenish yellow.
		Blue.	..	Yellowish orange.
		Grass Green.	...	Red.
		Yellow.	...	Violet.
		Pink.	...	Greenish blue.
		Crimson.	...	Green.
4TH ORDER	...	Blueish Green.	...	Pink.
		Yellowish pink.	...	Greenish blue.
		Red.	...	Green.
5TH ORDER	...	Pale blueish green.	...	Light pink.
		White.	...	Whitish.
		Pink.	...	Light Green.

The above sequence of colours is the same as that which holds in "Newton's rings"—*i.e.*, the coloured rings so often seen when

two 3 by 1 slips, or one such slip and a cover-glass, are pressed together so as to touch in the centre.

It is to be observed that the colours obtained with any thickness of selenite in the two positions of the analyser are always strictly *complementary* colours, since those colours which are best transmitted in one position are quenched in the other position, and *vice-versa*. This every microscopist knows, but it is most readily verified experimentally by placing a piece of Iceland spar over a hole in a piece of paper, laying a piece of selenite over the spar, and examining the two images with an analyser. The colours of the images will be seen to be complementary, but the part where they overlap will still appear white (Fig. 2).

The effect of the thickness of an object on its colour is well shown in a slide of plaited horsehair. Where two hairs cross we get a colour quite different from that of either hair.

When two selenites are placed one above the other, we get a different colour from that of either selenite seen separately. A second selenite, placed with its optic axes parallel to the corresponding axes of the first, will evidently have the same effect as increasing the thickness of the first selenite by that of the second. On the contrary, if the second is turned through a right angle so that its optic axes are now perpendicular to the *corresponding* axes of the first—(*i.e.*, so that the axis of the *extraordinary* ray in one selenite is parallel to the axis of the *ordinary* ray in the other)—it will, so to speak, hurry up those components of the waves that had got most behind in the first selenite. The effect is the same as if a slice of the same thickness as the second selenite had been removed from the first.

By rotating one selenite into different positions over the other, we can get the colours corresponding to any thickness of selenite not greater than the sum nor less than the difference of the thicknesses of the two selenites employed. This principle is used in the selenite stages which accompany most of our more expensive microscopes.

The Black Cross seen on starch-grains is familiar. A similar cross is seen on transverse sections of animal hairs, plant scales, especially those from the leaves of certain species of the order *Eleagnaceæ*—such as our Sea Buckthorn (*Hippophaë rhamnoides*),

certain structures occurring in the elytra of beetles, such as the cockchafer and large water-beetle, the bordered pits in a section of deal wood, etc. If tartaric acid—or, better, salicine—is properly crystallised on a slide, the crystals will radiate outwards from one or more common centres, and a very fine black cross will be seen under the polariscope.

This black cross is due to the fact that a doubly-refracting object exhibits no polariscopic appearances when it is placed with either of its two optic axes parallel to the direction in which the light is polarised. Consider, for example, a stellate hair formed by a number of hairs rotating from a common centre (Fig. 7). Let the light be polarised in the direction of the arrow. The two hairs (*I, I*) are pointing parallel to this direction, and the hairs (*U, U*) are perpendicular to the same direction. Each of these four hairs has *one* optic axis parallel and the other perpendicular to the direction of polarisation; therefore, all four hairs appear dark on the dark ground. The four hairs (*E, E, O, O*) have their optic axes inclined at 45° with the direction of polarisation, and they are therefore in the most favourable position to show polariscopic appearances. The intermediate hairs also show polariscopic effects, which are, however, rather less marked.

The spines of certain Echinodermata are sometimes mounted arranged in a radiating pattern, and the same effect is well shown in such a slide, notably in “professional” mounts of the anchors and plates of *Synapta*.

When a number of plant-hairs combine to form a scale—as in the *Elæagnaceæ*, above mentioned—the effect is to give a distinct cross. The same is true of the crystals of salicine. Here one of the optic axes of each crystal points towards the centre, from which they radiate, and the black cross is formed by those crystals, in which this optic axis is either parallel or perpendicular to the direction of polarisation.

A starch-grain is built up of concentric layers, which are gradually added to it as it grows. A perfectly round starch-grain would grow directly outwards from its centre in just the same way as the salicine crystals grow. It would, therefore, exhibit a perfect cross in the form of two black straight lines intersecting at right angles in the centre of the grain. As a matter of fact, however,

most starch-grains grow more or less unevenly, one side growing faster than the other ; consequently, the black cross is more or less unsymmetrical, both in form and position.

Colours of Starch-Grains seen with Selenite.—When any of the objects mentioned in the last section are viewed with selenite, they appear coloured, but *the colours do not form a cross*. Instead, the object appears to be divided into four quadrants, and one pair of opposite quadrants is differently coloured to the other pair.

Now, this appearance agrees exactly with what we should expect from theory. In a round object of this class there will be one diameter— $O O'$ (Pl. VII., Fig. 8)—along which the optic axes of the object are parallel to the corresponding axes of the selenite. Here the colours will be the same as we should get by slightly *increasing* the thickness of selenite (just as in the case of two selenites placed with their axes parallel). Along the perpendicular diameter (E, E) the optic axes are perpendicular to the corresponding axes of the selenite, and the colours are the same as we should get by slightly *decreasing* the thickness of the selenite. Between these two diameters there must evidently be a pair of diameters ($I I, U U$), along which the colour of the object is the same as that of the selenite, and these divide the object into four quadrants, two of which exhibit the colours of rather *thicker* and two those of rather *thinner* selenite than that employed in its examination.

Conclusion.—The reader cannot fail to be struck with the remarkable way in which the appearances seen with the micro-polariscope fit in with the wave-theory of light. It has hardly been possible in the present paper to give more than the barest outline of the theory of polarised light, and I have purposely omitted any reference to polarisation by reflection, rotatory polarisation, many other phenomena which, though equally interesting to the physicist, are not so familiar to the microscopist. For these I must refer the reader to Mr. Spottiswoode's book and other more technical works on the subject.

The more deeply Polarised Light is studied, the closer becomes the agreement between experiment and the wave-theory. For the present, however, I can only conclude by expressing a hope that

my readers will have derived some small insight into the wonderful molecular mechanism which gives rise to the beautiful colours and crosses that are revealed by the microscope with the aid of polarised light.

Hop-Pickers' Ophthalmia.*

Abstract of a Paper by PERCY T. ADAMS, M.R.C.S., D.P.H.,
Resident Med. Officer Kent County Ophthalmic Hospital.

FOR the past six years, during the latter part of August and throughout the month of September, cases of an acute form of ophthalmia have occurred in Kent among the hop operatives.

A peculiarity of the disease lies in the apparent immunity of men; women and children are more intimately associated with the actual handling of the hop-cones in plucking them from the bine. Men engaged in the hop industry are, however, subject to the disorder. The disease is associated with no particular plantation, growth of hops, encampment, or village. It is mostly prevalent at the time of the maturity of the catkins only, and does not appear to be infectious. All, whether "home-pickers" or strangers, appear to suffer equally from the disorder, which appears to partake of a partly mechanical origin.

In two cases which came under the writer's notice, the patients positively affirmed that the advent of their symptoms followed shortly after an accidental rubbing of their eyes with hop-soiled hands. I made observations of the operatives whilst actually engaged in hop-picking, and noticed that the women, whilst bending over the bines, frequently applied their hands to their foreheads to brush aside the hair from the face. A woman with a much-inflamed eye who came to the hospital maintained that it came on shortly after rubbing "some of the stuff from the hops" into her eyes from her hands.

Patients complain of an acute smarting pain, which becomes

* From *British Medical Journal*, May 13, 1893. Our thanks are due to the Editor for kindly lending the electros.

rapidly worse. This stinging sensation I have known to affect the skin as well as the eye after a stroke from a hop-bine alone. The hands are often much soiled and blackened with the resinous matter from the hops, and the odour of the volatile oils is very noticeable about the patients' clothing.

The usual mode of introduction of an irritant from the hop appears to be by the hands. The older agricultural labourers say that, prior to the introduction of lever presses for compressing the dried hops in the "pockets," this work was performed by treading

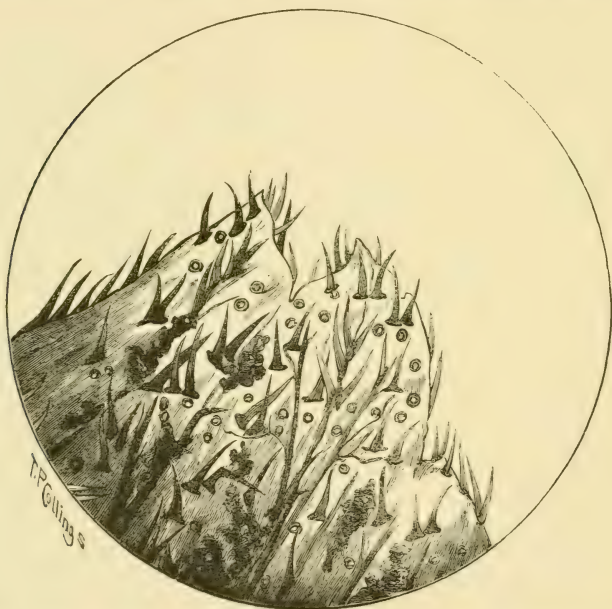


Fig. 68.—Hair-like appendages upon the bracts of the hop-catkins, magnified. them in with the feet. This created a considerable quantity of dust; and it was not an unusual occurrence for their eyes to become affected from the hop-dust. Since the application of these presses, this inconvenience no longer occurs.

MICROSCOPICAL EXAMINATION OF THE HOP PLANT.

On examining the bracts, leaves, and bine, thorn-like, hairy processes are seen on all, those upon the bine being larger and

coarser than those upon the leaves and bracts. These sharply-pointed processes are scattered all over the surface of the bracts, with the apices of each spine directed towards the distal end of the bract as regards its point of attachment to the pedicel. The spines on the leaves are confined more to the edges, and do not appear to be so hard and dense and sharp as those upon the bracts. In Fig. 70 some of the characteristics of these spinous processes are shown.



Fig. 69.—Spine-shaped hairs of the hop-bracts. *a*, Entire spinous process ; *b*, fractured ; *c*, showing hollow central canal (transverse section).

In staining with various dyes, the outer portion of the hair accepts the staining re-agents less readily than the softer internal parts. When for any reason they are fractured between the faceted point of attachment to the bract and their pointed extremity, the fractured ends are not unlike those of a mature bone or a dry branch of a tree, with a less dense central portion. The bracts are also covered with glandular structures and lupulinic grains. Emphasis is placed on the fact that these hairs are denser

and sharper in structure in the mature hop-catkin, because in this fact lies, I believe, the explanation of this disease of hop-pickers, and one of the reasons why it is most prevalent at the hop-harvest.

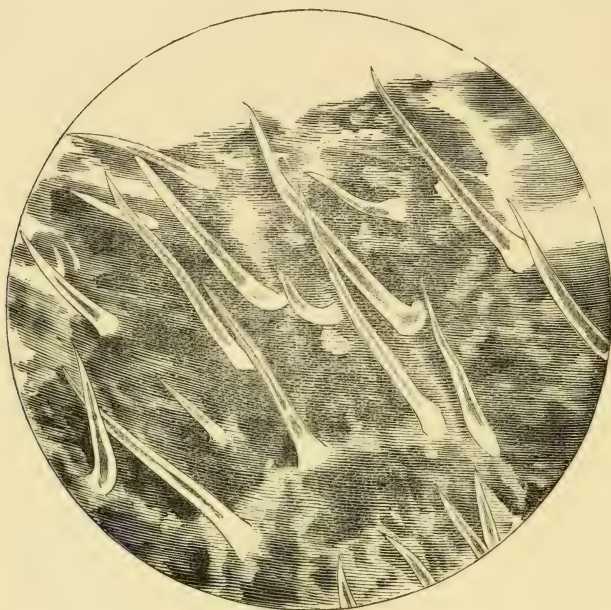


Fig. 70.—A microphotograph of the spines upon the bracts of the hop.

PROBABLE MODE OF PRODUCTION OF THE OPTHALMIA.

Belonging as the *Humulus lupulus* does to the same family as the *Urtica urens*, or common stinging nettle, and the order URTICACEÆ (which also includes some very severe stinging foreign specimens), and knowing that the hop-plant possesses those sharply-pointed appendages, is it not probable that this painful affection, which is produced immediately and often continues to become worse, is explained by the introduction, either by movements of air, by gravitation, or upon the hands of the hop-pickers, of some of those spinous processes of the hop-plant, which, becoming impacted into the conjunctiva or cornea, form the initial cause of the disease? It is also probable that upon them the volatile and resinous matters, etc., of the hop itself, or even

micro-organisms, are introduced, which may vary and modify the subsequent features of the trouble. The principal signs are primarily those of a mechanical irritant in this disease. The employment among the operatives of glass protective spectacles,

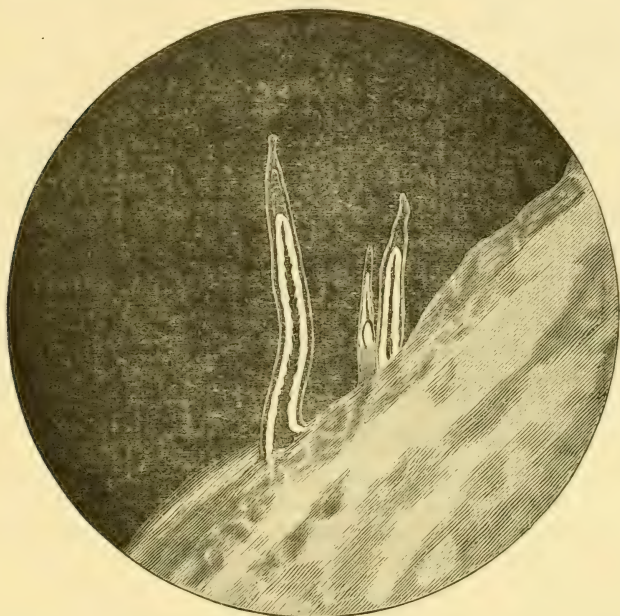


Fig. 71.—A microphotograph of the spinous hairs of the hop-plant.

and the use of gloves during the operation of hop-picking, to be abandoned at once on the termination of the work, together with more personal cleanliness, are most important prophylactic measures, though difficult to enforce.

The following is given in *Science* (April 28th, 1893) as the *best* formula for Müller's fluid :—

Bichromate of Potassium	2 per cent.
Sulphate of Sodium	2 „
Water	96 „

In practice it is convenient and sufficiently exact to dissolve 2 grammes of each salt in 1,000 cubic centimetres of water.

The *Leucosporæ*.*

IN this section of the Agaricinæ, the spores are typically white, although in some species there is a more or less decided but very faint tinge of yellow or pink. The commonest spore-form is elliptical and with a smooth epispore, although in the genera *Lactarius*, *Rassula*, and *Laccaria*, the spores are subglobose and minutely warted or echinulate. This section contains more species than all the other sections of the AGARICINÆ added together, and, as would be expected, presents the most complete sequence in the differentiation of the sporophore. *Lenzites*, with its corky pileus and gills, connects with Polyporeæ through *Dædaleæ*. The genera—characterised by a tough, leathery pileus, that dries up and becomes rigid and persistent, as *Panus*, *Lentinus*, etc.—are but scantily represented in Britain or even in Europe; but on the other hand, are more numerous in the tropics than the fleshy, putrescent genera, which attain their maximum, both in development and numbers, in the north temperate zone.

In the accompanying illustration (for the use of which we beg to thank Messrs. George Bell and Sons), the following species are represented:—

- Fig. 1.—*Hygrophorus Wynnii* and section of same, natural size.
 „ 2.—*Lactarius blennius*, about one-third natural size, and section of same, natural size.
 „ 3.—Spores of same, $\times 400$.
 „ 4.—*Cantharellus aurantiacus*, about two-thirds natural size.
 „ 5.—*Nyctalis asterophora* and section, two-thirds natural size.
 „ 6.—*Lentinus tigrinus*, about two-thirds natural size.
 „ 7.—*Panus stypticus*, natural size.
 „ 8.—*Lenzites flaccida*, two-thirds natural size.
 „ 9.—Section of same, natural size.
 „ 10.—*Omphalia telmaticea*, small specimen, natural size.
 „ 11.—Section of same, natural size.
 „ 12.—*Pleurotus gadinoides*, natural size.
 „ 13.—Section of same, $\times 2$.
 „ 14.—*Clitocbe ericetorum*, half natural size.
 „ 15.—Section of same, half natural size.
 „ 16.—*Xerotus degener*, natural size.
 „ 17.—*Schizophyllum commune*, natural size.
 „ 18.—Section of gills of same, showing the split margin.
 „ 19.—*Trogia crispa*, small specimen, natural size.

* From the *British Fungus-Flora*, by George Masser (in three vols.). Vol. II. (London: George Bell and Sons. 1893.)

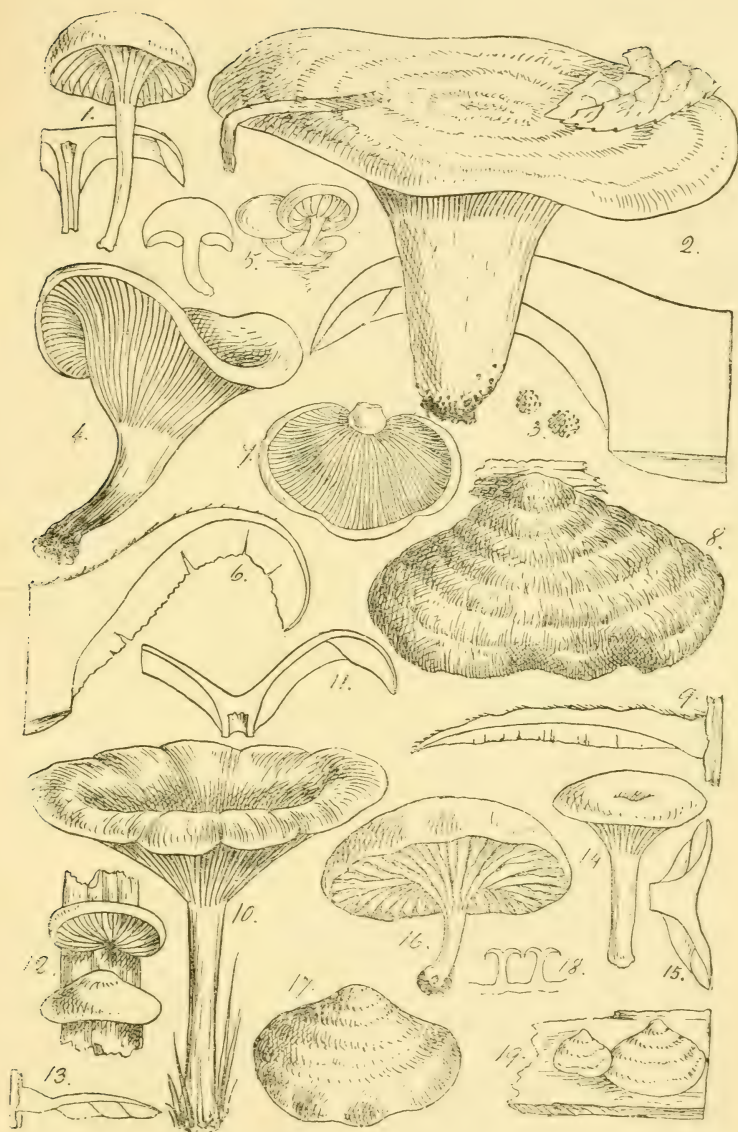


Fig. 72.—LEUCOSPORÆ.

Stains and Staining, as applied in the Examination of Animal and Vegetable Structures under the Microscope.*

BY PETER WYATT SQUIRE, F.L.S.

INTRODUCTION.

I AM glad to have the opportunity of giving my first lecture on "Staining" in this theatre, as I owe my initiation into histology to two veterans of this Society. My first tuition in cutting sections was from Mr. Thomas Greenish, and it was through Professor Bentley that I entered the Biological Laboratory at King's College. About three years ago I thought it would be useful, both to physicians and pharmacists, if a few of the more general methods and formulæ for microscopic work were inserted in the *Companion*. But on reference to the various standard works on microscopical technique, it was found that the whole subject was in a semi-chaotic condition:—Different formulæ under the same name, the same formulæ under different names, varied with printers' errors, wrong copying, and mistranslations.

Even when a formula could be traced back to an authentic source it was sometimes worded in such a vague, compound-it-as-you-please fashion, as to be little more than a suggestion. Examples of this will be noticed later on. This rendered necessary a considerable amount of experiment—partly chemical, partly microscopical—with a view of getting the most effective staining solution in its most permanent form.

As the work involved a more intimate knowledge of animal histology and pathology than I personally possessed, I was fortunate in obtaining the assistance of Mr. F. J. Warwick, M.B. (Cantab.), formerly Demonstrator of Bacteriology at King's Coll.

Some of the results have been already published, and it is in reference to these and to a few more recent developments that, at the invitation of your President and committee, I will now speak.

* Read before the Pharmaceutical Society of Great Britain at an evening meeting in London, Wednesday, February 8, 1893. From *The Pharmaceutical Journal*, by kind permission of the Editor.

NUCLEAR STAINS.

As in both animal and vegetable sections it is generally the nuclei which form the geographical landmarks of the structure, the most important class of reagents which are used in any of the branches of microscopical work, are the "nuclear stains."

They are so called because they stain the nucleus more strongly than the rest of the structure, and when certain precautions are adopted the differentiation can be made so complete that the nuclei will be strongly stained in an otherwise colourless section. Some sections illustrating this will now be projected on the screen.

There are several nuclear stains, the most important of which is hæmatoxylin, and when a good solution is used the results are excellent.

Logwood and Hæmatoxylin.

In the early history of microscopy, various preparations of logwood were used, but these have been practically discarded in favour of the more definite crystallised hæmatoxylin. The literature of this stain is very confusing and contradictory. Not only do the various writers differ amongst themselves as to its merits and demerits, but it happens occasionally that curious contradictions occur in the same book.

In one of the best books on staining (published in 1890) the chapter on hæmatoxylin contains the following remarks:—

"None of the solutions are perfectly stable; only one or two are fairly so. In general, freshly prepared solutions stain badly and diffusely; they ought to be allowed to ripen before use. This takes, according to the nature of the solution, a few hours, or days, or months. On the other hand, kept solutions generally go bad by precipitating or becoming acid or becoming mouldy. Most of the solutions, when in good staining order, have a great tendency to overstain. The stain is fairly permanent in balsam, but is sure to fade a little, and may fade a great deal."

This would be enough to prejudice any beginner against hæmatoxylin, were it not that on the next page, under Delafield's solution, it is stated:—"The solution keeps well, it may be said for years. It is extremely powerful, and when properly used is very precise." A few pages further on in the same book

is found under Ehrlich's hæmatoxylin, "After ripening it will keep with a perfectly constant staining power for years. Sections are stained in a few minutes. The stain is very appropriate for staining in bulk, as overstaining does not occur."

Many formulæ have been written for hæmatoxylin solution, but there are only three of them—Delafield's, Kleinenberg's, and Ehrlich's—which are of general interest. In all three, alum is present as one of the ingredients; the idea being that the alumina forms with the colouring matter an insoluble "lake," and so acts as a mordant.

Delafield's Hæmatoxylin.

In this there is nothing characteristic, except the large proportion of alum to hæmatoxylin and the use of methylic alcohol (wood spirit) in the place of rectified spirit. It is rendered in some of the books as methylated spirit, which, however, possibly answers just as well.

As an instance of the same formula appearing under different names, Lee, in his "Microtomist's Vade-Mecum," gives the following interesting history connected with Delafield's solution:—"It had long been in use in the Institute of Pathology at Heidelberg, where it was communicated by Pfitzner to Flemming, who published it and particularly recommended it. Flemming then attributed the formula to Grenacher; and, in consequence, the stain went for years by the name of 'Grenacher's hæmatoxylin.' In 1885 matters were set right by Prudden explaining that the stain was the invention of Delafield and publishing the correct formula. This accounts for the same formula appearing in different books as Delafield's and as Grenacher's."

Kleinenberg's Hæmatoxylin.

Great differences exist in the wording of the formula given for this solution. That given by Foster and Balfour in their *Elements of Embryology*, published about twenty years ago, has been published without comment into most of the text-books.

A.—Make a saturated solution of crystallised calcium chloride in 70 per cent. alcohol, and add alum to saturation.

B.—Make a saturated solution of alum in 70 per cent. alcohol.

C.—Add 1 part of *A* to 8 parts of *B*.

D.—Make a barely alkaline saturated solution of hæmatoxylin in water. For staining, place eight or ten drops of *D* in a watch-glass half filled with *C*.

The peculiarity of this modification is the presence of a quantity of chloride of calcium in the solution. Its general use is for staining in bulk, and the idea is that the presence of soluble salts in the staining fluid will set up diffusion currents between it and the fluids in the mass to be stained and so aid the penetration of the stain. So far well, but when we come to add alum to the solution we are at once met with double decomposition between the alum and the calcium chloride, resulting in the precipitation of sulphate of lime.

The extent of this decomposition depends partly on time and agitation, but principally on the condition of the alum. Here is a bottle of the saturated chloride of calcium solution in which alum crystals have been shaken at intervals during the whole of the day, and the decomposition is visibly very slight. Here, on the other hand, I have a quantity of the chloride of calcium solution, to which I add *powdered* alum instead of crystals, and shake. In a few minutes it will be a solid mass. Which of these two modifications is intended by the inventor of the solution and the various book-writers who have copied his formula?

It would appear from the *Quarterly Journal of Microscopical Science* of 1879 that Kleinenberg himself used the following process:—"Prepare a saturated solution of calcium chloride in 70 per cent. alcohol, with the addition of a little alum; after having filtered mix 1 volume of this with 6 to 8 volumes of 70 per cent. alcohol. At the time of using the liquid, add as many drops of a concentrated solution of hæmatoxylin in absolute alcohol as are sufficient to give the required colour to the preparation of greater or less intensity according to desire."

In this later form the saturation of the alcohol with alum is omitted, but as alum is soluble in 70 per cent. alcohol, only about 1 in 1000, this can make little or no difference. But as the addition of "a little alum" to an unknown quantity of saturated solution is still far from definite, I have suggested the following formula:—

Kleinenberg's Solution (Improved Formula).

Hæmatoxylin, $2\frac{1}{2}$ grammes ; crystallised calcium chloride, 20 grammes in 10 c.c. of distilled water ; alum, 3 grammes in 16 c.c. of distilled water ; rectified spirit, 240 c.c. Dissolve the calcium chloride and the alum in their respective quantities of water by the aid of heat ; mix the solutions and immediately dilute with rectified spirit ; after an hour filter and add the hæmatoxylin. This makes a good working solution which keeps well. Of course, it contains the alumina in solution not as alum, but aluminium chloride. If in special cases the colour is considered too strong, the dilution (when staining in bulk) must be made with some of the solution to which hæmatoxylin has not been added.

Ehrlich's Hæmatoxylin.

This differs from the other two forms, in being strongly acidified with acetic acid.

The usual book formula is hæmatoxylin, 2 grammes ; absolute alcohol, 100 c.c. ; glycerine, 100 c.c. ; distilled water, 100 c.c. ; glacial acetic acid, 10 c.c. ; "alum to saturation," 2 grammes being the maximum quantity retained in solution at the ordinary temperatures.

It is generally admitted that when freshly made, Ehrlich's solution is next to useless, and we are told that it takes months, if not years, to "ripen." The fact is that the rapidity depends upon exposure. One method is to allow the solution to lie exposed in an open dish till its volume is reduced to half, which gives it, of course, the benefit of concentration as well as oxidation.

It is, however, on absorption of oxygen that the whole question of hæmatoxylin ripeness practically depends ; and, as this is accelerated in alkaline and greatly retarded in acid solutions, it is obviously more rational to effect the change upon the hæmatoxylin *before* rather than after conversion into Ehrlich's solution.

An "ammoniated hæmatoxylin" was therefore made by exposing to the air a solution of hæmatoxylin in proof spirit made alkaline with carbonate of ammonia, and afterwards converted into Ehrlich's solution in the usual way. A staining fluid made on these lines may be used at once and found superior to a normal Ehrlich's solution which may have been ripening in the orthodox

fashion for the last ten years. Since the mention of this "ammoniated hæmatoxylin" in my "Methods and Formulæ" there has been some talk of using hæmatein instead of hæmatoxylin in Ehrlich's solution. This is, of course, the natural suggestion, but commercially hæmatein is a very variable product, frequently next to useless for staining purposes, and I have yet to meet with a commercial sample even of ammoniated hæmatein capable of staining nuclei with such a depth of rich colour and definition as an ammoniated hæmatoxylin prepared on the lines I have indicated. The formula at present stands thus:—

Ammoniated Hæmatoxylin (Squire).

Hæmatoxylin, 15 grms. ; ammonium carbonate, 3 grms. ; proof spirit, 300 c.c. ; place in a large bottle and shake at intervals for three days, leaving the stopper out between the shakings. Allow the solution to evaporate to dryness in an open dish at the temperature of the air, and (substituting the crystalline product thus obtained for hæmatoxylin in the ordinary formula) dissolve in the following mixture:—Absolute alcohol, 750 c.c. ; glycerine, 750 c.c. ; distilled water, 750 c.c. ; ammonia alum, 15 grms. ; glacial acetic acid, 75 c.c.

Colour Produced by Hæmatoxylin.

Hæmatoxylin solutions stain the nuclei violet, and in order to change this into blue it is usual to soak the sections in water taken from the house supply (not distilled water), but as the alkalinity of the water varies in different localities, a better and more uniform result is obtained by using a weak solution of bicarbonate of sodium ($\frac{1}{2}$ grain to the ounce).

With the view of testing whether strong daylight and alkali were essential to the formation of the blue, I placed some sections in glazed earthenware covered pots with distilled water recently boiled in platinum. The violet colour gradually became blue, but there is no doubt that the change is much assisted by an alkali. After the blue colour is developed, if the sections are not for immediate mounting, they should be kept in the dark, and in 70 per cent. alcohol. If kept in water they fade rather quickly when exposed to daylight, and are bleached by direct sunlight.

Carmine.

Carmine is also used as a nuclear stain, and the two solutions more generally employed are Grenacher's alcoholic borax carmine and Orth's lithium carmine. Under ordinary circumstances they act as general stains, affecting the ground tissue as well as the nuclei. By subsequent treatment with acidulated alcohol, or acidulated glycerine, the colour is discharged from the ground tissue without seriously affecting the nuclei. Used in this way, carmine becomes a good nuclear stain. It should be remembered that the sections must not be washed in pure water, as the colour will be to a great extent removed, nor in acidulated water, as the carmine will be precipitated on the sections. Alum carmine and alum cochineal are useful nuclear stains not requiring after-treatment with acid. I now add chloroform to the lithium carmine and alum carmine solutions, to give them the keeping properties of chloroform water.

Picro-carmines.

These have been largely used, and, in some quarters, are great favourites. Ammonia, lithia, or soda, is used as the solvent for the carmine, and a certain quantity of picric acid is added. Several complicated formulæ have been published for ammonia picro-carmine; I have tried the following, amongst many others, and find it to be the best all-round solution.

Ammonia Picro-carmine.

Carmines, 1 gramme; strong solution of ammonia, 3 c.c.; distilled water, 5 c.c. Dissolve the carmine in the ammonia and water with a gentle heat, then add saturated aqueous solution of picric acid, 200 c.c.; heat to boiling, and filter.

Picro-Lithium Carmine.

Some workers prefer the picro-lithium carmine, and one of them told me that he preferred to use the following proportions: Lithium carmine solution, 100 c.c.; saturated solution of picric acid, 270 c.c.

ANILINE NUCLEAR STAINS.

There are several aniline dyes which are used for nuclear staining:—methylene blue, methyl green, safranine, gentian violet.

vesuvine, fuchsin, and Hoffman's blue. I have stained a series of sections with the various nuclear dyes (for comparison), which will now be projected on the screen.

The usual process is to stain $\frac{1}{4}$ or $\frac{1}{2}$ per cent. aqueous solutions and wash in methylated spirit. Methylene blue and methyl green have the reputation of being so readily washed out in the methylated spirit as to be worthless. I found that this was partly true, but also that it could be obviated by washing the sections (when removed from the stain) in distilled water, previous to the differentiation in methylated spirit. Treated in this manner, the nuclear staining is very beautiful. This also applies to Hoffman's blue, and partly to vesuvine; with the latter, however, it is not a necessity. Safranin and gentian violet worked better by transferring the sections directly from the stain into 90 p. c. alcohol.

Contrast Stains.

Very frequently other dyes are used to stain the ground a colour which is a good contrast to that employed for the nuclei. Brown, orange, or pink are used after nuclear blue or green; carmine red is generally counterstained yellow or indigo blue, and fuchsin red, as in tubercle bacilli, is counterstained with nuclear blue. It is important that the ground stain should be made weaker than the principal stain, so that the whole tissue may be shown without detracting from the nuclei or bacilli, as the case may be. The following colours are used as counterstains for animal sections, but they are not so appropriate to vegetable work:—benzopurpurine, eosin, erythrosin, orange, acid rubin, and picric acid. Solutions of these are on the table.

As examples of specific stains may be mentioned fuchsin, methylene blue, and gentian violet for bacteria; osmic acid for fatty elements; victoria blue and rose bengal for demonstrating elastic fibres; methyl violet, iodine, and safranin for amyloid degeneration.

Methylene Blue.

This is probably the most powerful of all the aniline dyes, and one of the most variable in composition, as well as most erratic in solubility. I had hoped that the experiments, which are now in progress with a view to explaining these peculiarities, would

have been so far complete as to enable me to place the results before you to-night. The investigation has, however, proved more lengthy than I anticipated, and I am obliged, reluctantly, to hold over this part of the paper for another occasion.

Iodine Green.

Iodine green, or methyl-green, has long been used as a reagent for amyloid, in apparent ignorance of the fact that the reaction really was due to methyl-violet, contained as an impurity in the iodine green. It is exceedingly difficult to obtain a green quite free from violet. As far as I know, there is not a single maker at the present time who can supply it; but I have here a sample obtained some years ago by Dr. Warwick, which is practically pure, and with which the amyloid violet cannot be produced. The presence of violet in these greens can be very simply demonstrated. Here are samples of iodine green and methyl-green dissolved in water; to these will be added sufficient caustic alkali to decompose and so decolorise the green, when the residual violet will be plainly seen.*

CELLULOSE REACTIONS.

After the nuclear stains, probably the most important reagents to the worker in botany are those which affect cellulose and its modifications. Pure cellulose is coloured yellow by iodine, the colour being changed to a blue on the addition of slightly diluted sulphuric acid (about 2 volumes of strong acid to 1 of water), or a strong solution of chloride of zinc.

Schulze's Solution.

This solution, containing iodine, iodide of potassium, and chloride of zinc, gives a violet reaction with unaltered cellulose, and yellow with lignified cellulose.

In working out the best formula for Schulze's solution some rather peculiar points were noticed, which are sufficiently interesting to be placed on record. One book-formula reads as follows: Zinc is dissolved in hydrochloric acid; the solution is allowed to

* This reaction is the same for both greens, which really differ only in name. Formerly it was customary to use methyl iodide in the manufacture of this dye, hence the names "methyl-green," and "iodine green" is rather a misnomer, although both names are still used by the dealers.

evaporate in contact with metallic zinc, until it attains the thickness of a syrup; the syrup is then saturated with potassium iodide, and subsequently with iodine. Another formula on the same lines directs the evaporation to be stopped at the consistence of strong sulphuric acid, which is a long way short of a syrup. A third formula starts from dry (fused) chloride of zinc, and adds water, but in none of these formulæ is there any hint that the slightest difficulty is likely to be experienced in carrying out the directions.

The first difficulty likely to be encountered is with the zinc chloride. When a neutral solution of chloride of zinc is evaporated to a syrupy consistence it loses hydrochloric acid, becomes basic, and precipitates oxychloride on being largely diluted with water. When evaporated to a solid and fused, this dissociation is very much greater; if, in addition, the evaporation has been carried out as directed over an excess of metallic zinc, the product contains such a quantity of oxide that the addition of water results in a mud rather than a solution. The pharmacopœia process for making liquor zinci chloridi is on exactly the same lines, with the result that on dilution one dispenser sends out a muddy solution, another filters and sends out a clear one, a third adds hydrochloric acid, and the whole results in a correspondence in the *Chemist and Druggist*.

The proper remedy is to neutralise the liquor zinci chloridi with hydrochloric acid before making up to the final volume; the point taken being either when the solution ceases to precipitate on being diluted with ten volumes of water, or when this diluted solution just reddens methyl orange.

Supposing that we start with a fairly neutral solution of zinc chloride, and try to saturate it with potassium iodide, one of three things will happen, according to the strength of the solution. If very strong, sp. gr. 2.2 (syrupy), it will scarcely dissolve any of the salt, and although a quantity is taken up on heating, and the solution is quite permanent, it has positively no staining power whatever. If to four of the syrupy be added two of water, the potassium iodide dissolves more freely, but in a day or two the bottle becomes half filled with crystals (principally a double chloride of zinc and potassium). If, however, to three of the

syrupey fluid two of water be added, no crystallisation takes place with the potassium iodide, and the solution answers every requirement. At this point it agrees in consistence and sp. gr. with strong sulphuric acid, about 1·84. If much weaker than this it will dissolve potassium iodide to almost any extent, but the solution, when finished, stains the cellulose such an ugly brick-red colour, that as a re-agent it is next to useless.

It might readily be supposed that the iodide of potassium only acted as an aid to getting sufficient iodine into the solution; but there is no doubt that it modifies largely the colour produced on the cellulose. When only traces of iodide are present the colour produced is a pure blue; with increasing quantities of iodide the colour becomes more and more violet. The amount of iodine which the solution is capable of dissolving depends upon the strength of zinc chloride and the proportion of potassium iodide, but there is no advantage in adding more than 0·1 per cent. The final result therefore is :—

Chlor-zinc Iodine (Improved formula).

Zinc chloride solution (sp. gr. 1·85)* 70 c.c. : potassium iodide, 10 grammes; iodine, 0·1 gramme. The solution can only be used as a reagent and not as a dye. Structures stained with it cannot be mounted in any of the ordinary mounting media. I have kept them for a short time by mounting them in some of the fluid, and ringing the preparation with caoutchouc cement.

Cellulose can be stained permanently by carmine, hæmatoxylin, nigrosine, methylene blue, safranine, and fuchsin.

I use Grenacher's alcoholic borax carmine undiluted, and my ammoniated hæmatoxylin, or Delafield's solution, diluted 1 to 9 of distilled water. The aniline dyes are used in dilute aqueous solutions, containing one-eighth or one-fourth per cent. of dye.

When the cellulose undergoes the change known as lignification, its reactions are altered. It is coloured yellow by chlor-zinc iodine, red with phloroglucin, yellow by aniline chloride. The

* A solution of this sp. gr. may be obtained by evaporating 100 c.c. of liquor zinci chloridi B.P., remembering that the sp. gr. of this liquor should be about 1·53, and not 1·46 as officially stated.

two latter are much assisted by hydrochloric acid. The results of these reactions also cannot be preserved in the usual mounting media.

Sections containing mixed tissue, partly unaltered cellulose and partly lignified, give very striking results with the aniline dyes, with this additional advantage, that the preparations showing the reactions can be preserved for years.

DOUBLE STAINING.

When a section is passed through methyl green solution and afterwards carmine (the minute details I have already published), the lignified portion is coloured green, and the unlignified red. Acid green may be used in the place of methyl green with a like result. When picric acid is used with carmine, nigrosine, or Hoffman's blue, the picric acid dyes the ligneous portion, and the others colour the unlignified structure red, black, and blue respectively.

Sieve-areas.

These are well shown by Hoffmann's blue and eosin, also, but not so strikingly, by hæmatoxylin. Sections representing these combinations will be projected on the screen, and I will bring under your notice the various points as they occur.

I have now concluded the matter which I wished to put before you, and first I have to thank you for the patient hearing which you have given me, and then to express my gratitude to Mr. Curteis who is handling the lantern, and to my assistant, Mr. Stewart, both of whom have given me great assistance in the preparation of this lecture.

Insects are the lowest animals known to assist in seed dissemination. Mr. Darwin tells us of locust excrement containing seeds which grew when planted. Considering that locusts often occur in vast swarms, they can hardly fail to be highly effective agents in seed dissemination, thus repaying to some extent for the immense damage they often do.

—*Popular Science Monthly.*

Zoology of the Invertebrata.*

“THE last few years have witnessed a great extension in our knowledge of the structure and relationship of the Invertebrata. The earth has been ransacked for new forms, and improvements in microscopes and in technique have facilitated a more minute and thorough examination of these forms in the laboratory. This increase in our knowledge has necessarily been accompanied by a re-arrangement of material ; many intermediate forms have been discovered, and unexpected relationships have been revealed, and these have entailed a revised classification.”

In the handsome volume before us Mr. Shipley has given such an account of the Invertebrata as cannot fail to be most helpful to the student. He describes in a very careful manner, at least, one or more examples of the larger groups, and gives, generally, a shorter account of the most interesting modifications presented by other members of the group. He treats his subject more particularly from a morphological standpoint, touching but lightly on the Histology, Embryology, and Natural History of the forms described.

The author states in the Introduction that “the organic world has developed in two diverging directions, one corresponding to the animal and the other to the vegetable kingdom, and though there is no difficulty in distinguishing the higher forms of these two kingdoms, it is often by no means an easy matter to determine whether some of the lower forms should be grouped with the plants or with the animals ; hence any scheme of classification is dependent on individual opinion. There are a number of characters which, if met with in an organism, would justify us in classing it as an animal ; but in many cases one or more of these animal features are absent, and, again, other features may be

* “ZOOLOGY OF THE INVERTEBRATA : A Text-Book for Students.” By Arthur E. Shipley, M.A., Fellow and Assistant Tutor of Christ’s Coll., and Demonstrator of Comparative Anatomy in the University of Cambridge. 8vo, pp. viii—458. (London : Adam and Charles Black. 1893). Price 18s. net.

present, which, as a rule, are only found in plants, so that it becomes at once evident that the line between animals and plants, at any rate in their lowest forms, represents no scientific frontier, but is an arbitrary boundary which is apt to be shifted, now forward, now backward, according to the opinion of the investigators."

The volume is divided into twenty-one chapters, each treating of a separate class of the Animal Kingdom, commencing with Protozoa, Metazoa, Coelenterata, etc., up to Insecta, Arachnida, and Chordata.

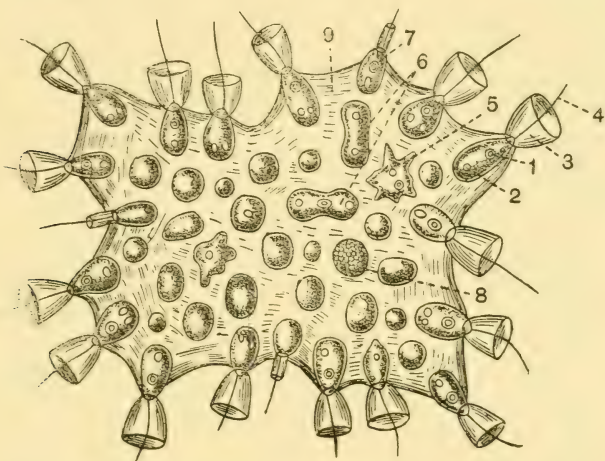


Fig. 73.—*Proterospongia Haeckeli*, Sav. Kent, $\times 800$.

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| 1. Nucleus. | 6. Other individuals undergoing fission |
| 2. Contractile vacuole. | 7. Individual with collar contracted. |
| 3. Collar. | 8. Individual divided up into number of spores (microgonidia). |
| 4. Flagellum. | 9. Jelly-like supporting matrix. |
| 5. Amoeboid individual sunk in supporting jelly. | |

The Protozoa have been divided into two groups: the Gymnomyxa, corresponding with the old group, Rhizopoda, and the Corticata, which comprise the Infusoria and Gregarinidea. We shall select our first illustration from the group Corticata. These animals have, as a common feature, a differentiation of the protoplasm into a more fluid central portion and a firmer cortical

layer usually associated with a limiting membrane, which surrounds their body and gives it a definite shape. As a consequence of the presence of this cortical layer, these forms, which take solid food, have acquired one or more channels through which the nutriment is ingested, and usually a definite area whence the undigested remnants are extruded.

The genus *Proterospongia*, Fig. 73, one of the Flagellates discovered by Saville Kent, in which the individuals of the colony are sunk in a jelly, lends some support to the view that Sponges may have originated from colonies of Choano flagellata.

Most Flagellata live in fresh water; some are marine, and some parasitic, living in the alimentary canal or blood of Vertebrates and Arthropods.

Turning to the Metazoa we abstract a short description of the sponges.

Fig. 74.—Part of a section through *Grantia labyrinthica*, vertical to the margin, and to the two surfaces of the wall of the cup. After Dendy.

1. Inhalent pore.
2. Exhalent canal.
3. Inhalent canal.
4. Cavity of flagellate chamber.
5. Pore area.
6. Gastral skeleton.
7. Dermal skeleton.
8. Tubar skeleton.
9. Embryos.

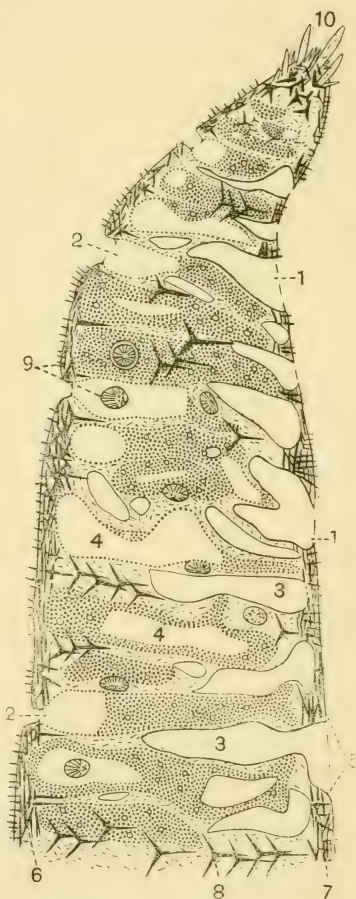


Fig. 74.—*Grantia labyrinthica*.

The substance of the sponge is composed of three layers: the

ectoderm, the endoderm, and between them the mesoderm. The ectoderm consists of flattened cells, covering the outside of the sponge, and lining certain pits or depressions which are pushed into the substance of the sponge, and are termed inter-canal spaces. The openings from the exterior into the inter-canal spaces are termed pores. The inter-canal spaces open on their inside by numerous apertures, into the flagellate chambers. These flagellate chambers are the most characteristic feature of Sponges; the flagella keep up a constant current of water which passes in at the pores, through

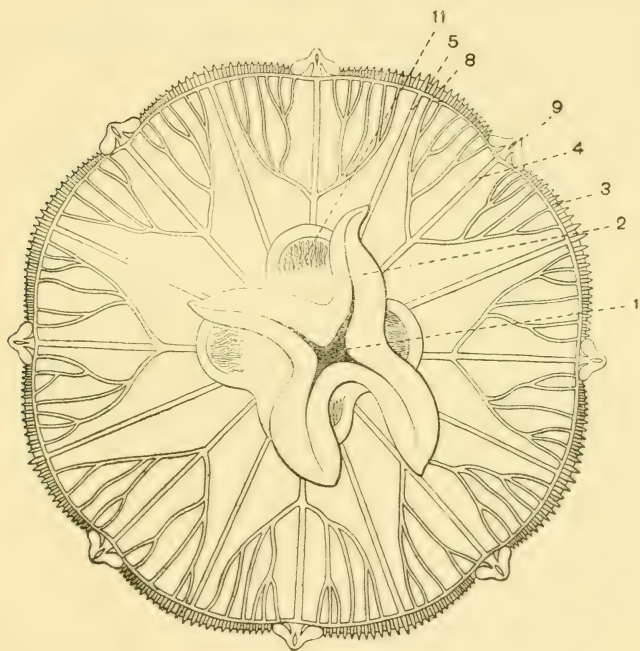


Fig. 75.—*Aurelia aurita*.

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|---|--|
| 1. Mouth. | 5. An adradial canal. |
| 2. Circumoral perradial processes. | 8. The circular canal. |
| 3. Tentacles on the edge of the umbrella. | 9. Marginal lappets hiding tentaculocysts. |
| 4. One of the branching perradial canals. There are four of these, and four similar interradial canals. | 11. Gastral filaments. |

the inter-canal spaces and flagellate chambers, and into the central cavity, and leaves through the oscula.

From Coelenterata we select *Aurelia*, Fig. 75, a Scyphomedusan very commonly met with round our coast, swimming on the surface of the sea.

The mouth of *Aurelia* is surrounded by four perradial processes, and the manubrium is short. The sense organs are modified tentacles, which bear endodermal otocysts and ectodermal pigment spots, or eyes. An aboral and an oral pit, both lined by specialised epithelium on the surface of the disc, are regarded as olfactory.

In the order HEXACTINIA belonging to this division are the MADREPORARIA. They are solitary or colonial; their most remarkable characteristic is their power of secreting a calcareous skeleton. The Madreporaria are of the greatest importance in the history of the earth. They are the true corals, and their skeletons form by far the greater part of the coral rock which has built up a considerable portion of the earth's crust. Reef-forming corals do not, as a rule, grow below the forty-fathom line, and are not usually found north or south of a belt extending 30° each side of the equator.

Chapter VI. treats of PLATYHELMINTHES, which are divided into three classes: Turbellaria, Cestoda, and Trematoda. The Turbellaria are subdivided into Rhabdocoelida and Dendrocoelida. In the latter division are found the genera Tricladia and Polycladia. We have selected Fig. 76, showing Plan of a Polyclad.

In the Polyclads there are many, usually eight, nerve cords, which diverge from the central cerebral ganglion (Fig. 76). Sensory cells, provided with tactile hairs, occur in the ectoderm. The eyes are usually two or four in number, but may be more numerous, and they increase by division. Auditory vesicles also occur, they are often single, and consist of a vesicle full of fluid, in which a calcareous otolith floats. The anterior end of the body is remarkably sensitive, and in some genera forms a tactile proboscis, which can be retracted into a sheath.

To the third class of this division, the TREMATODA, belong the Liver Fluke, *Fasciola (Distoma) hepatica*, (Figs. 77 and 78), and is found in the liver of diseased sheep. It is about three-quarters of an inch long, and has a flattened, leaf-like shape.

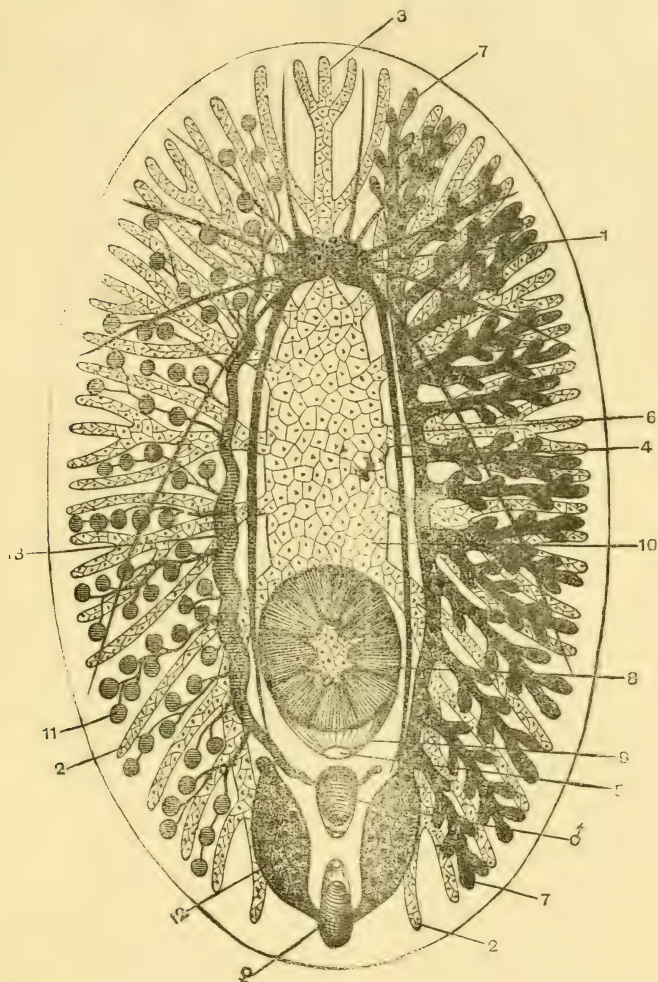


Fig. 76.—Plan of Polyclad. After Von Graff.

1. Brain.
2. Intestinal branches.
3. Anterior unpaired branch.
4. Longitudinal nerve cord.
5. Mouth.
6. Oviduct.
7. Ovarian follicle.
8. Pharynx.
9. Pharyngeal pouch.

10. Stomach.
 11. Testicular follicle.
 12. Uterus.
 13. Vas deferens.
 - ♂ Male copulatory organ, with the male aperture behind.
 - ♀ Female copulatory organ, with the female aperture before it.
- The eyes are omitted.

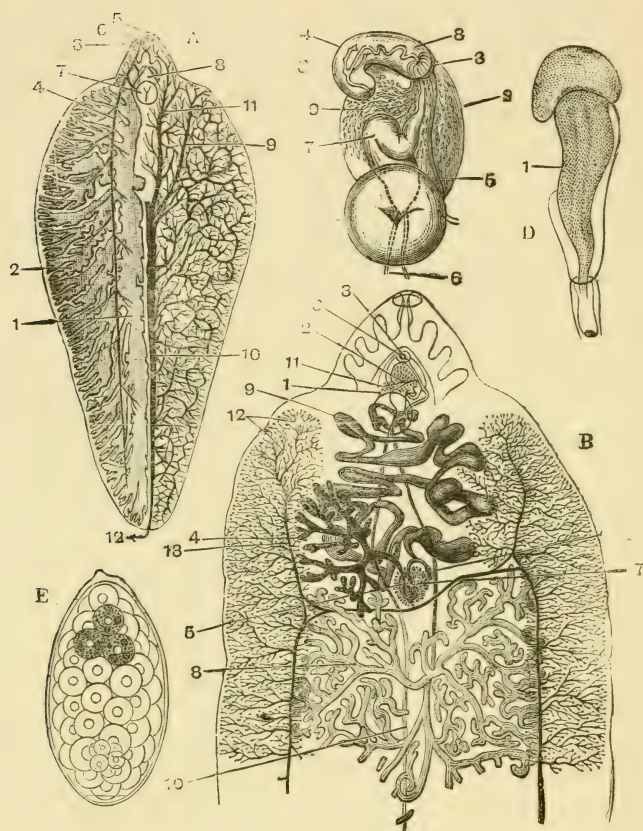


Fig. 77.—*Fasciola hepatica*.

A.—*Fasciola hepatica*, from the ventral surface ($\times 2$).

The alimentary and nervous systems only shown on the left side of the figure, the excretory only on the right.

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| 1. Right main branch of the intestine. | 7. Ventral sucker. |
| 2. A diverticulum. | 8. Cirrus sac. |
| 3. Lateral ganglion. | 9. Left anterior dorsal excretory |
| 4. Lateral nerve. | 10. Main vessel. [vessel. |
| 5. Mouth. | 11. Left anterior ventral trunk. |
| 6. Pharynx. | 12. Excretory pore. |

B.—Anterior portion, more highly magnified.

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|--------------------------|--|
| 1. Cirrus sac. | 8. Anterior testes. |
| 2. Ductus ejaculatorius. | 9. Uterus. |
| 3. Female aperture. | 10. Vasa deferentia. |
| 4. Ovary. | 11. Vesicula seminalis. |
| 5. Oviduct. | 12. Yolk gland. |
| 6. Penis. | 13. Its duct (from Marshall and Hurst,
after Sommer). |
| 7. Shell gland. | |

C.—Genital sinus and neighbouring parts.

- | | |
|-------------------------------------|-----------------------------------|
| 1. Ventral sucker. | 6. Vasa deferentia. |
| 2. Cirrus sac. | 7. Vesicula seminalis. |
| 3. Genital pore. | 8. Ductus ejaculatorius. |
| 4. Evaginated cirrus sac (? penis). | 9. Accessory gland (from Sommer). |
| 5. End of vagina. | |

D.—A ciliated internal end from the excretory apparatus.

1.—Orifice of the flame cell (highly magnified).

E.—Egg of *Fasciola hepatica*, $\times 330$ (from Thomas).*Explanation of Fig. 78 (page 302).*

- | | |
|---|---|
| 1. Nearly ripe cecariæ. | 14. Lips of redia. |
| 2. Cystogenous cells. | 15. Collar. |
| 3. Daughter rediæ. | 16. Processes serving as rudimentary
feet. |
| 4. Limbs of digestive tract. | 17. Embryos. |
| 5. Head papilla. | 18. Trabeculæ crossing body cavity of
redia. |
| 6. Eye-spots. | 19. Birth opening. |
| 7. Same, degenerating. | 20. Morulæ. |
| 8. Germinal cells. | 21. Oral sucker. |
| 9. Cells of the anterior row. | 22. Ventral sucker. |
| 10. Embryo in optical section, gas-
trula stage. | 23. Pharynx. |
| 11. Pharynx of redia. | All from Marshall and Hurst, after
Thomas. |
| 12. Digestive sac. | |
| 13. Oesophagus. | |

Accompanying these two illustrations we have a good description of Liver fluke, with a most interesting account of its life-history, of which we regret our space will not allow us to make an extract. It has been computed that each fluke produces half-a-million eggs.

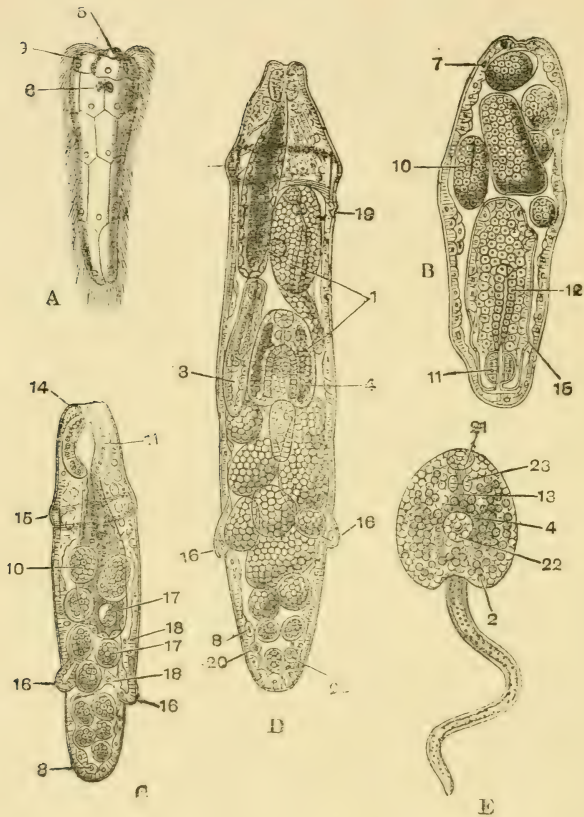


Fig. 78.—Five stages in the life-history of *Fasciola hepatica*, all highly magnified.

A.—The free-swimming embryo.

B.—The sporocyst, containing young rediæ.

C.—The young redia, the digestive tract shaded.

D.—An adult redia, a daughter redia, two cercariæ, and germs.

E.—The free cercaria. The figures have the same significance throughout, and are described on preceding page.

We will now pass on to Chapter XV. and take an example from the ECHINODERMATA, which are divided into five classes, viz. : Asteroidea, Ophiuroidea, Crinoidea, Echinoidea, and Holothuroidea. From the last of these we have selected.

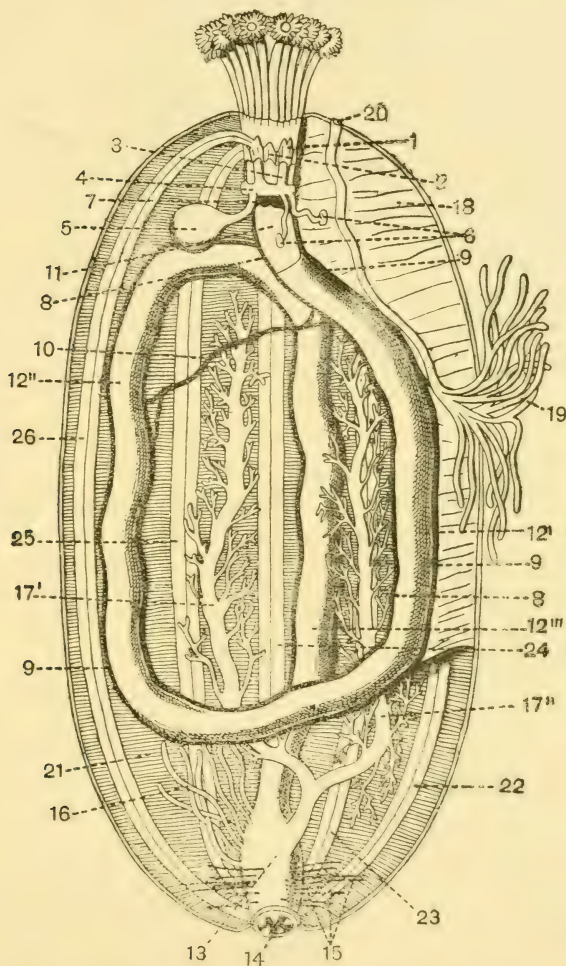


Fig. 79.—View of the internal organs of a Holothurian, which has been cut open along the middle dorsal line. From Leuckart.

Explanation of Fig. 79 (page 303).

- | | |
|---|--|
| 1. Radial ossicle of the calcareous ring, into which the longitudinal muscle is inserted.
2. Interradial ossicle of the calcareous ring.
3. Radial water-vascular vessels.
4. Circumoral ambulacral ring.
5. Polian vesicle.
6. Two stone canals ending in madreporic plates; the upper one is attached to the dorsal mesentery, the lower one hangs freely.
7. Circumoral blood-vessel.
8. Ventral blood-vessel.
9. Dorsal blood-vessel.
10. Anastomosing branch between different parts of the ventral blood-vessel. | 11. Anterior part of alimentary canal.
12. 12' 12'' The three limbs of alimentary canal.
13. Cloaca.
14. Cloacal opening with five teeth.
15. Radiating muscles of cloaca.
16. Organs of Cuvier.
17' 17'' Respiratory trees.
18. Dorsal mesentery, with free posterior margin.
19. Generative organs.
20. Opening of generative duct.
21. Circular muscles in body-wall.
22. Right dorsal muscle.
23. Right ventral muscle.
24. Medium ventral muscle.
25. Left ventral muscle.
26. Left dorsal muscle. |
|---|--|

The tentacular ampullæ are omitted; the mouth is in the centre of the divided tentacles.

Chapter XVI. treats of the ARTHROPODA, which are divided into two large groups, according to the nature of their breathing organs:—The BRANCHIATA, which breathe by gills and are typically aquatic; and the TRACHEATA, which breathe by tracheæ or lung books, and are typically terrestrial.

The BRANCHIATA include but one class, the CRUSTACEA, and as a last specimen of these beautiful illustrations we have selected Fig. 80, *Gammarus neglectus*, an animal very nearly allied to the Fresh-water Shrimp, which is well-known to our readers. It belongs to the sub-order AMPHIPODA. These crustaceans are generally small, but some few of them living in Arctic Seas, or at great depths in the ocean, attain several inches in length. They inhabit both salt and fresh water, and progress by swimming or jumping. The males may usually be distinguished by the development of their olfactory hairs on the first antennæ, by the absence of oostegites, and by the presence of strong prehensile hooks on the anterior thoracic feet.

The student of Zoology cannot fail to derive a large amount of valuable information from a careful study of this volume. It contains 263 very fine engravings. Those which we have selected are by no means the best; but they are such as we thought would prove most interesting to our readers generally.

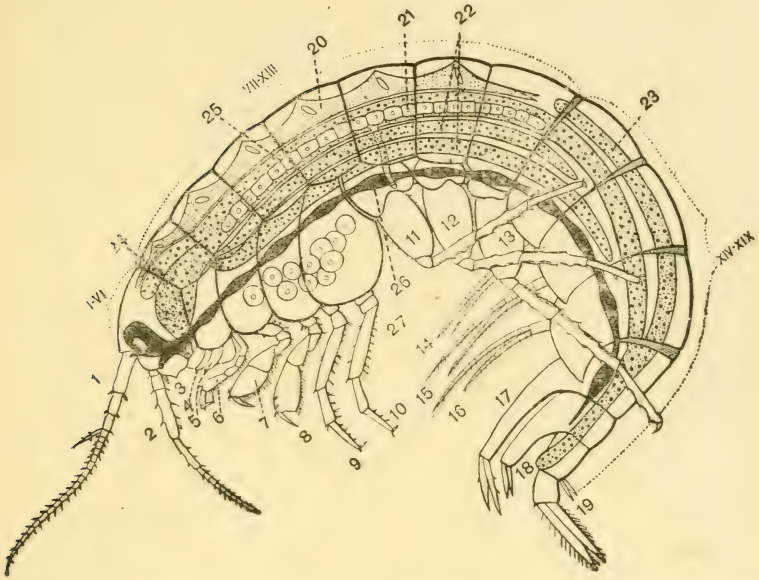


Fig. 80.—*Gammarus neglectus*. Female bearing eggs seen in profile.
From Leuckart and Nitsche, after G. O. Sars.

I.—VI.—Cephalothorax.

VII.—The thoracic segments.

XIV.—XIX.—The six abdominal segments.

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|---|---|
| 1. Anterior antennæ. | 20. Heart, with six pairs of ostia. |
| 2. Posterior antennæ. | 21. Ovary. |
| 3. Mandibles. | 22. Hepatic diverticula. |
| 4. First Maxillæ. | 23. Posterior diverticula of the alimentary canal. |
| 5. Second Maxillæ. | 24. Median dorsal diverticulum. |
| 6. Maxillipede. | 25. Alimentary canal. |
| 7—13. Thoracic limbs. | 26. Nervous system. |
| 14—16. Three anterior abdominal limbs for swimming. | 27. Ova in egg pouch, formed from lamellæ on the coxæ of the three anterior thoracic limbs. |
| 17—19. Three posterior abdominal limbs for jumping. | |

The description of each section of the animal kingdom is prefaced by a Zoological Chart, or "Scheme of Classification." There is also a good Index.

Our best thanks are due to Messrs. Adam and Charles Black for so liberally allowing us the use of the illustrations.

Boiling Water and Rotifers.

BY C. O. SONNTAG.

A SHORT time ago I happened to come across a green, slimy-looking pond near a farm, which I proceeded to examine with the enthusiasm and joyous anticipation peculiar only to a microscopist. I filled several bottles with the rather unpleasant looking fluid before me, and examined it as soon as I got home. It was swarming with what I naturally expected, namely, *Euglena*, *Vorticella*, *Amæba*, *Brachionus*, *Hydatina*, *Stentor*, and a legion of ciliated *Infusoria*. It was indeed a rich find, and at once the idea struck me to seize this opportunity, seeing the material was next to inexhaustible, to try my hand in fixing the organisms in the bottles, and make some permanent slides of them. The specimens represented by the largest number were the Rotifers *Brachionus* and *Hydatina*, and I resolved to experiment exclusively upon them, although other organisms would naturally also come in (accidentally) for their share. I examined a few drops in a watch-glass, and noticed that the Rotifers were gorged with food, and therefore not only unpleasant, but unsuitable for careful examination and mounting purposes. I allowed the bottles to stand over night. This allowed all floating extraneous particles to settle to the bottom, and the water became thus tolerably clear, revealing to me next morning not only hundreds, but thousands of busy Rotifers dancing near the surface of the water like so many grains of silversand.

Through the pocket lens I saw that they were still full with food-particles, and I thought of several means of how to clean them and make them more transparent, but the process was naturally to be a "wholesale one" if it was to be of any use at all. I knew that the use of chemicals was excluded, as these would take more out of the transparent Rotifers than desirable, and would even spoil them altogether. But I thought a good and reliable plan would be to starve them for a day or two, and I carefully poured them from the field bottles into clean water, let them stand for two days, examined them every morning, noon, and night, and was pleased to notice that they gradually became

whiter, and on the third day they were in a very desirable condition. But what was I to do with them now?

I placed a few in a watch glass and added osmic acid. It certainly fixed them, but they lost their natural transparency, the ganglionic region became contracted as by a rope, the cilia were unsatisfactory, and the trophi and intestines not very clear. I tried others with corrosive sublimate, which seemed to darken them and affect them more or less like osmic acid. Next I experimented with tannic acid (10 per cent. in water), and found that the cilia stood out very clearly, whilst the rest of the body became blurred and tanned. Picro-sulphuric acid fixed only a few specimens satisfactorily, whilst chloral hydrate, acetic acid, alcohol, and chromic acid were likewise more or less unsatisfactory. I also experimented with chloroform, and found that, after allowing them to revive, they would never re-expand, their tails swimming about in a contracted or crippled form, in which condition they were, of course, useless.

This was the whole list of fixing fluids that I was practically acquainted with, and as none had given me entire satisfaction in the above experiments, I looked about for some others. I happened to go to my chemical press to review the list of all the deadly poisons at my command, when I heard the tea-kettle singing cheerfully on the kitchen fire, and at once I thought of making use of the boiling water. Quickly I got half a dozen tumblers with concave bottoms *in the inside*, poured about 3 or 4 tea-spoonfuls of the water containing the starved Rotifers into each tumbler, and next poured *quickly* and *suddenly* a solid stream of boiling water into each tumbler. I let the water cool, gave it then a circular motion by passing a pencil around the inside of each tumbler, as this gathered all the killed organisms into the centre of the *concave* bottom, and then examined a drop of the sediment in a watch-glass.

My surprise was immense. The trochal disc was better defined than with any chemical excepting tannic acid: the trophi were quite clear, also the œsophagus, stomach, gastric glands, ovary, contractile vesicle, and foot-glands. The longitudinal muscles stood out clear like polished ropes, while the vascular system could be easily traced. The ganglionic ring was indistinct.

whilst other nerve-cells seemed to appear where I had not expected any, unless I mistake them for organs that I know nothing about. So far I was pleased, but I thought the trochal disc should be brought out in a less swollen but more distinct condition, and as I knew the effect of tannic acid upon the ciliary wreath, I added half a tumbler of tannic acid (10 per cent. in water) to every tumbler of cold water. I boiled it, poured it upon my starved Rotifers in the same way as described above, and find now that every organ has kept its natural position and outline. The cilia are quite clear and straight, and the internal organs very distinct. What a pleasant discovery this was for me !

I at once proceeded to stain them with various dyes, of which saffranine has given excellent results, but it must be used in a very weak solution to avoid over-staining. I cannot say very much about the use of multiple staining (so as to increase the differentiation), but I hope to find some way of doing it too. To mount them permanently very *shallow* cells are needed, so as not to crush the delicate bodies. To prevent the appearance of fungoid growth I add a drop or two of carbolic acid to every tumbler containing the water with the fixed Rotifers. As it was impossible to separate the Rotifers from the *Protozoa* which were floating about in the water, they, of course, had to undergo likewise the hot-water bath, and showed the same satisfactory result if tannic acid was added to the water ; without it, the *Vorticellæ*, *Stentor*, and most ciliated *Infusoria*, swelled out and became unrecognisable. *Euglena* and *Amæba* behaved particularly well. I also mounted some in pure glycerine, and to transfer the organisms from pure water to the denser medium I placed the *stained* animalcules and Rotifers in a test-tube, covering them with a little carbolised water. When they are all settled to the bottom, I gently pour pure glycerine *down the side* of the test-tube, put in a cork, and let the tube stand. The glycerine will sink to the bottom, and the stained Infusoria and Rotifers will sink very gradually from the water into the glycerine. This is my experience of the use of boiling water as a fixing agent ; I have also tried it for fixing *Spirogyra*, *Cladoptera*, *Zygnenia*, *very young leaves*, and *ovaries*, in all of which the protoplasm has not shrunk in the least, whilst the nucleus and nucleolus stood out very clearly.

Bacteriological Value of Corrosive Sublimate as a Germicide.

BY PROF. V. A. LATHAM, D.D.S., CHICAGO UNIV.

THE following is from an abstract of a Thesis by Charles T. McClintock, A.M., candidate for the degree of Ph.D. (Univ. of Mich.):—

“In 1881 Koch recommended corrosive sublimate as the most efficient of all known substances for disinfecting purposes. Since then it has been universally used. After making some hundreds of experiments, the author finds that Koch and those who have confirmed his work based their conclusions on faulty experiments, the most important of which was the failure to notice that the sublimate formed, with the gelatinous coat of bacteria, a compound insoluble in water, but soluble in salines, and readily removed by the blood. When bacteria treated with sublimate were transferred to gelatine or agar, the capsule of mercury compound prevented the growth of the germ, and the false conclusion was drawn that it was dead. In the author's experiments this capsule of mercury was removed by precipitation with hydrogen sulphide; while Koch states that all bacteria are killed in a few minutes by solutions of sublimate, 1 : 1000, the experiments show the bacteria—such as *Staphylococcus pyogenus aureus*—may grow after having been in the 1 : 1000 solution ninety-three hours; 1 : 100, eleven hours; saturated solution, one hour.

Bacillus subtilis grew after lying in 1 : 1000 forty-one hours; saturated solution, eighty-five minutes; typhoid germs, after 1 : 1000, one hour; germs in fæces, after twenty-four hours in a saturated solution. Several experiments comparing strong vinegar with 1 : 1000 sublimate indicated that they have the same germicidal power. The experiments also indicate that the larger number of bacteria in a given culture are comparatively easily killed, and that the ratio of those killed by a germicide to those that survive is of no value. The conclusions drawn from the experiments are :

(1) That the high rank heretofore given corrosive sublimate as a germicide is without warrant and was based on faulty experiments.

(2) Different cultures of the same germ may vary largely in their resistance to germicidal agents.

(3) Corrosive sublimate forms with cellulose, with silk, with albuminous bodies, and with some parts of bacteria, a compound that cannot be removed without washing. When acting on a germ it forms a capsule around the germ, which protects it from the further action of the germicide, and in turn presents the growth of the germ unless removed. But this capsule may be removed by the solutions, as in the blood. The action of sublimate on bacteria is probably closely analogous to that of alcohol, etc.

(4) The presence of a gelatinous envelope in many, if not all, bacteria has not received due attention from writers on this subject.

(5) In albuminous fluids—and practically all disinfection has to do with such—corrosive sublimate of any strength whatever is not a reliable germicide.

(6) While sublimate has no great germicidal powers, it does not follow that it may not be a valuable disinfectant. This point remains to be proved.*

A question relative to the subject of germicides and disinfectants is the correct definition and meaning of the words. With some authors they both occupy the same place and have no distinction. If the new dictionaries are consulted, a great diversity of opinion will be found; in others, no distinction amongst them at all. Let us ask our readers to consider the agents known as antiseptics, and compare their action with the germicidal agents and the deodorisers, and with disinfectants and antizymotics, and the difference between the process of fermentation with that of putrefaction. To understand the agent's action we must know its classification, its position in the drug list, its value as a specific agent, its use and its composition, and certainly its meaning.

Drugs classified from a practical standpoint and not a purely scientific, as the actions of few drugs are well known.

Antiseptics (*αντι, ἐηπιω*, to make putrid) are agents which prevent the decomposition of organic substances, and destroy infec-

* This is a most important question in the field of Bacteriology, Surgical Pathology, and Clinical Surgery and Medicine, and is worthy of the attention of every man who aims at bacteriological work, and we hope these experiments may be continued and further worked out.—*Ed.*

tion and fœtid odours, thus arresting fermentation. Examples are : Creosote, carbolic acid, peroxide of hydrogen, bichloride of mercury, etc.

Disinfectants.—Agents capable of depriving foul odours, etc. of their germs by chemical affinity. Examples : Chlorine, charcoal, HCl, carbolic acid, etc.

Deodorisers.—Agents which act chemically, capable of destroying infectious and fœtid odours, but forming new compounds with the products of decomposition. Examples :—Eucalyptus, iodol. carbolic acid, etc.

Detergents.—Those agents which cleanse wounds, ulcers, etc., and act as stimulants and emollients. Examples :—Borax, burnt alum, tragacanth, etc.

Germicides.—Agents which destroy any form of microbe or disease germ and prevent the formation of microbes. Examples : Cassia oil, permanganate of potash, HgCl_2 , H_2O_2 , etc.

Antizymotics are agents which prevent putrefaction and are divided into disinfectants and antiseptics.

Disinfectants are divided into Deodorants, Detergents, and Germicides.

The use of the agent depends on how it is used with the disease, and should be classified in that way. Say, in treating some disease, we wish to arrest the same and to destroy the conditions existing in the wound, we use the drugs above in the following order :—

Deodoriser.—To cleanse and purify.

Germicide.—To kill microbes if present.

Detergent.—To cleanse by acting as a stimulant and increase vigour of the tissue.

Disinfectant.—Keeps away the odours of the germs.

Antiseptic.—Prevents the decomposition of the organic constituents and destroys infection, arrests fermentation, etc.”

It is a suggestive fact that hooked fruits occur on low plants, never on trees ; also that geologic time hooks appeared simultaneously with land animals.

Leaves from my Note-Book.

Geology in the Making.

BY MRS. ALICE BODINGTON.

A SPECIAL interest attaches to any phenomena going on under our own eyes or in our own times that show those processes still in action, which have so curiously shaped and carved the crust of our earth in past ages, and those which have preserved or destroyed animal and vegetable forms.

When Hugh Miller discovered his wonderful fish of the Old Red Sandstone, he described the *Pterichthys* and other fossil forms as having apparently died in agony, with outstretched and stiffened fins, as though overwhelmed by some great cataclysm ; perhaps, some volcanic upheaval of the sea-bottom. I have come across a description in the *Amer. Naturalist* for Aug., 1892, which forcibly reminds me of Hugh Miller's theory of sudden destruction.

The history of the Tile-Fish (*Lopholatilus chamaeleonticeps*) is among the strangest known :—" So far as we have any information no one, fisherman or naturalist, ever saw a tile fish until March, 1879, when a Gloucester (U.S.) fishing schooner took 6,000 lbs." " In 1880 and 1881, a few were taken by the U.S. Fish Commission steamer. In March and April, 1882, vessels arriving in American ports reported passing through large numbers of dead and dying fish off the southern coast of New England and Long Island. Vessels reported sailing for forty to sixty miles through floating fish (in one instance, through one hundred and fifty miles), so that it became evident that a vast destruction had taken place. It was estimated that an area of 5,000 to 7,000 square statute miles were so thickly covered that the total numbers must have exceeded a billion. The next year the Fish Commission searched in vain for these fish on the ground where they were formerly so abundant, and no one has since reported a specimen." If this mysterious destruction had occurred near land, and the myriads of dead fish had been gradually covered, we should have much the same condition of things as that imagined by Hugh Miller.

A curious piece of contemporary geology is being worked out

in New Jersey.* The whole coast has been long sinking, and the process is still going on. A curious industry is carried on in the southern part of the State—the mining for cedar. Some of these noble trees, exhumed from their swampy burial, exceed three feet in diameter, with the timber perfectly sound. “The ‘lay’ of these uprooted trees indicates the devastation probably of extraordinary cyclones occurring at immense intervals of time, thus levelling one forest upon another that had been thrown long before.” The cedars growing there to-day send their roots amongst their long-buried ancestors. The rings upon some of the exhumed trees show a growth of 1,500 or possibly 2,000 years, and the “existence of at least two buried forests beneath the present growth is indisputable.”

Dr. Lockwood, from whom I quote, says that on the south side of Raritan Bay is a clay bluff, which in his own recollection has lost much of its height. Standing on this bluff at times of very low tide, he has seen in the distance stumps of trees in the same position in which they were left by the woodman’s axe when he cut down the forest which grew on that bluff when it reached much further seaward than now. Nearer the shore could be seen a great number of broken bricks and a well-curb, the remains of a brick-yard, which, like the ancient bluff, had also gone to sea.

Dr. Lockwood, during a residence of many years at Keyport, not more than two miles from the bluff in question, had cherished a little grove of native saplings—scrub oaks, pines, persimmons, and gum-trees. Gradually these formed a dense covert, in which a number of singing-birds found shelter. But one summer evening a new visitor appeared whose delightful medley of song reduced all the other bird vocalists to silence. It was a mocking-bird (*Mimus polyglottus*), in former days common in the woods of New Jersey, but even forty years ago scarce, and now most rarely seen. The luxuriant forests in which it delighted have been felled, and with them the mocking-birds’ food and shelter. But this incident made Dr. Lockwood think of “interviewing” one of the oldest inhabitants of the district, a man born in the last century. All his life, he said, he had lived in these parts, and of late years he had heard no mocking-birds, but “plenty of ’em when I was a lad.

* Why the mocking-birds left New Jersey.—*Amer. Nat.* (Aug., 1892).

Many's the time I've gone nesting for them in the cedars that used to be yonder."

"What do you mean," said Dr. Lockwood, "by the cedars that used to be yonder?"

"On the bluffs over there," pointing to the bay. "Sixty years ago that bank was a great deal higher than now, and reached a sight further into the Bay. There used to be a thick forest of cedar on that bluff, and the mockers, a-plenty of them, built there every summer. But there's not been a single cedar there for many years—just how long I disremember. You see, *the bluff got going out to sea so fast they had to cut the cedars to save them*. You can see the stumps yet at any neap-tide. It 'most beats belief that the bluffs ever reached as far as them stumps. Why, in my time, a pretty good farm has gone off to sea. There used to be a brick-yard—that has gone off, too. It lay a little north of them cedars, and something can be seen of it when the tide suits. Old Auntie Willetts, now dead and gone, used to milk the cows alongside of what we call the black rock. That's gone, too, and I should think it has sunk considerable, for it's little more than the top of it that can be seen at neap-tide." Surely, an interesting bit of contemporary geology!

The old man knew nothing of subsidence; his theory was simple. "Naturally the sea was uprising, sort of overflow on the land. Wasn't it all the time getting the waters of all the rivers, without any let-up whatever?"

Not only does the present condition of New Jersey illustrate how profoundly a change of the flora of a district must affect its fauna, but in the peninsula of Sandy Hook it possesses a geological survival, which shows how rich the state must have been in plant and animal life when its coasts stretched further out into the warm waters of the Gulf Stream.

Whilst the mainland is suffering from subsidence of the land and denudation of the forests, Sandy Hook is increasing in both these respects. It is lengthening out without narrowing, and maintains, protected from the axe, a virgin forestage of the very tree flora which has so nearly departed elsewhere. So dense is the growth of cedars, with grand outliers of the crimson-berried holly, that not only are these evergreen groves rich in food, but they are

practically impervious to the winter winds. Here are rookeries of crows, which almost blacken the air as they return from their daily foraging. Here, too, are robins by the thousand. And here, too, in this bird paradise has *Mimus polyglottus* found a hospitable home. Summer and winter he stays, and with plentiful food and warm shelter he bids adieu to his migratory instincts. Such in former days we may believe was the condition of the virgin forests of New Jersey.

The Chemical History of Air.

AT the Sanitary Congress, at Portsmouth, Dr. W. J. Russell, the President, delivered an interesting address upon this subject, from which we extract the following:—"The properties and phenomena of air were to a great extent known to the ancients, but it was not until the seventeenth century was well advanced that an English chemist, Hooke, in 1665, discovered that what Boyle and the great Bacon thought a 'volatile nitre,' or a 'crude and windy' spirit in the air, was really oxygen. A hundred years later, Priestley re-discovered what Hooke and his successor, Mayhew, had already found out; but scholars had by that time become inoculated with the spirit of science, and there was no longer any fear that the truth would ever again be lost. Our means of analysing the gases of the air, and every other gas, remained comparatively imperfect until 1857, when Bunsen's great work on 'Gas Analysis' was published—a work which remains still a monument to its author's ingenuity and skill. But it was not until the second half of the nineteenth century had been advanced that the existence and importance of air-dust was suspected, and it was to the acuteness of Mr. Aitkin that we owed the discovery of how important a part air, pure and impure, would play in sanitary science.

He discovered air-dust in the spray from the sea, in the rarefied atmosphere on the summit of the Rijhi, impurities at the top of the Eiffel Tower, and dust in the fogs and mists. 'If there

were no dust in the air,' said Aitkin, 'there would be no fogs, no clouds, no mists, and no rain.' Our obligations to this discoverer were completed by his invention of the 'Koniscope,' a small and portable instrument for testing rapidly and easily the air in our cities and rooms—an instrument which every sanitary inspector would find of use. It was not to determine the absolute number of dust particles in the air that the Koniscope was chiefly used, but to compel them to tell by their behaviour and colour in a ray of light whether the dust particles present were few or many.

Mr. Aitkin thus describes his method of making impure air visible :—'The room is tested in every part, and the inside air gives, like the air outside, only the faintest colour. Three jets of gas are then lit in the centre of the room, which has the dimensions of 24 ft. by 17 ft. by 13 ft. Within thirty-five seconds of striking the match to light the gas, the products of combustion had extended to the end of the room, for the colours in the Koniscope had become dark blue; in four minutes the deep blue-producing air was found at a distance of two feet from the ceiling, and in ten minutes there was evidence of the pollution all through the room. It was strongly indicated near the windows, owing to the downward currents of cold air on the glass, and the impure currents could be traced to the floor and onwards to the fireplace, while a pure current could be traced from the door to the fireplace.'

It had been proved, however, that great epidemics like the cholera were not spread in their ordinary course by the air, for they did not travel faster than human intercourse, and it was fortunate for us that such was the case, but in certain circumstances—in the neighbourhood of infected spots, for instance—the air was known to be a medium of infection for short distances. If this half century had so extended our knowledge of nature, what may we not expect in the coming century?"—*Mon. Mag. Phar.*

For utilising the services particularly of mammals, many fruits have developed hooks or horns to catch in the fleece of passing creatures, who thus transport the seeds from place to place. An autumn tramp through our pastures will soon convince one of the efficiency of this mode of dissemination.

Microscopical Technique.

COMPILED BY W. H. B.

New Method of Preparing Dentine.*—In this method, suggested by Lepkowski, it is stated that sections of bone or dentine may be simultaneously softened and stained. The agent used is a modified form of Ranvier's fluid, and is composed of six parts of a 1 per cent. watery solution of gold chloride to three parts of pure formic acid. The pieces of teeth—which should be $\frac{1}{2}$ to $\frac{3}{4}$ mm. thick—are placed in this fluid for twenty-four hours; they are then removed, washed with distilled water, and placed in a mixture of gum arabic and glycerine for twenty-four hours. On removal from this last re-agent they are again washed with distilled water, then alcohol; after which they are embedded in celloidin or paraffin.

To Prevent the Reddening of Canada Balsam.†—The tendency of Canada balsam to become red may be checked, and the balsam bleached by the addition to the solution of a few crystals of pure metallic copper, precipitated from copper sulphate solution by any of the ordinary methods. This process originated with the late Allen Y. Moore, of Cleveland, O., and was the result of accident. What the philosophy of it is we do not know, as the copper crystals do not seem to be changed in any manner, even after long immersion in the solution of balsam.

An excellent Mounting Medium.‡—Dissolve gum dammar in benzole to the consistency of a thin syrup. Get rid of the larger particles of dirt by straining through an old silk handkerchief, and add to the cohalte about one-third of its volume of liquor potassæ. Shake until mixed, cork well, and set aside in a warm place for several weeks. On examination the mixture will be found to have separated into two layers, the lower of which (a resin soap) will contain all the impurities, the upper consisting of pure neutral dammar in benzole. Draw this off, and to each ounce add about eight to ten drops of poppy oil. This latter prevents the brittle-

* *Journ. Brit. Dental Assoc.*, xiv. (1893), p. 248.

† *National Druggist*, xxi. (1892), p. 196.

‡ *National Druggist*, xxi. (1892), p. 196.

ness which the dry dammar naturally possesses. The mounting medium thus prepared is far too thin for immediate use, but this is easily remedied by leaving the bottle open, or loosely corked, in a warm place for a day or two. If left open, cover the top of the vessel with a bit of lint cotton or a linen rag to keep out dust.

Structure of the Bacteria.*—Sjöbring has worked with the *Bacillus anthracis*, a hay bacillus, a vibrio, and several forms of cocci. He fixed the preparations by means of nitric acid, with or without alcohol, and stained with carbol-methylen blue or carbol-majenta red, afterwards decolouring with nitric acid and examining in glycerine and water.

Formula for making Picro-Carmine Stain.†—(1) Carmine, 1 grm.; liquor ammoniæ, 4 ccm.; mix and add 5 grms. picric acid. (2) Carmine, 15 grms.; picric acid, concentrated solution. Agitate the mixture (1) from time to time for two days, let it settle, decant, and evaporate decanted liquor at ordinary temperature, re-dissolve the dry residue in water, making 1 per cent. or 2 per cent. solution; filter when necessary. Triturate the carmine in water until very fine, add enough ammonia to dissolve the carmine, to this add slowly the concentrated solution of picric acid until the mixture has a blood-red colour, keep in a shallow dish until all odour of the ammonia has disappeared, filter, keep in a well-stoppered bottle, add a few drops of carbolic acid; filter before using.

Staining Small Organisms with picro-carmine and eosin.‡—A solution for staining small organisms with these stains can be made as follows:—Solution of a 1 per cent. of picro-carmine, 1 part; watery solution of a 2 per cent. of eosin, 1 part. The organisms to be stained should be left in the solution for from three to four days, and then washed in 70 per cent., and then in 90 per cent. alcohol.

Formula for Chloral Carmine.¶—Chloral carmine stain may be prepared by heating together on a water bath, for thirty minutes,

* *Centr. f. Bakt. u. Parasit.*

† *Journ. Brit. Dental Assoc.*, XIV. (1893), p. 248.

‡ *Journ. Brit. Dental Assoc.*, XIV. (1893), p. 248.

¶ *Journ. Brit. Dental Assoc.*, XIV. (1893), pp. 248—249.

half a gramme of carmine, 20 cc. of absolute alcohol, 30 drops of hydro-chloric acid, and then adding 25 grammes of chloral hydrate. The solution when cool is filtered.

A Pneumatic Bubble Remover.*—Mr. A. P. Weaver having been annoyed with air-bubbles in his mounts, has devised a simple air-pump for removing them as follows:—Take a small rubber syringe, the packing on the cylinder of which ought to be adjustable so as to fit the body of the syringe rather tightly, cut off the nozzle rather close to the body, and bore a hole 3 mm. in diameter, near the top of the latter, so that the packing will always be below the hole. Cut from an old rubber boot two washers 2.5 c.m. in diameter, and with a central aperture of 2 c.m.; cement these washers together with Red Cross cement (such as is used for mending punctures in pneumatic bicycle tyres); cut from the boot two more washers of the same outside diameter, and with a central hole a little smaller than the nozzle of the syringe; cement these last two washers together also, and cement them to the first two prepared; you will now have a shallow chamber a little larger than the cover-glass; force the nozzle of the syringe through the opening in the two top plates and firmly cement it there. All these joints must be air-tight. To use the instrument, place the slide on a smooth surface, wet the under surface of the rubber washers and apply the same to the slide, with the cover-glass in the shallow chamber. To make a good air-tight contact with the slide, grasp the syringe with the left hand and allow the lower side of the latter to hold the washers firmly to the slide. The hole drilled in the syringe is to act as a trap or valve, and is to be tightly covered with the first finger of the left hand (keeping the latter in position, grasping the syringe and holding the washer to the slide), at each downward stroke of the piston and uncovered at each upward stroke. This is, of course, done to prevent the entrance of air to the vacuum chamber beneath, after it has once been exhausted. I have found that three or four strokes are sufficient to bring all bubbles to the surface of the mounting fluid and cause them to burst.

* *The Microscope*, N.S., Vol. I. (1893), p. 41.

Method of Mounting Calcified Microscopic Specimens.*—

Mr. J. Mansbridge finds the one great disadvantage in the use of fluid balsam as a mounting medium for calcified specimens, where it is advisable to retain the air in the structure for purposes of clear definition, is the liability to run into any spaces, such as lacunæ or tubuli that may exist in the tissue. To overcome this difficulty he uses desiccated balsam in the following way:—Take a clean slide, place it upon a hot table with a small lump of balsam upon it, and cover with a hot cover glass, which must be pressed down in such a way as to expel all air from beneath it. Remove the slide to a cool surface and continue to keep pressure upon the cover glass for a few minutes, when the balsam will be found to be quite hard and the specimen ready to be labelled and put away finished. He finds the advantages are:—1.—There is no chance of the mounting medium running in and spoiling the section, as it becomes perfectly hard a few minutes after removal from the hot table. 2.—The specimen is finished at the time and is ready for the cabinet. There is no need to use a clip, no fear of the cover glass shifting if the slide is placed upon its side. 3.—It is very convenient for teaching purposes, as the ordinary stiff balsam soon becomes in a most deplorable condition.

Cedar-wood Oil.†—This oil possesses a growing importance in connection with its use with optical instruments. For this purpose it is essential that its refractive index should coincide as nearly as possible with that of the lenses with which it comes in contact, and it is usually necessary to condense the oil to some extent. Schimmel's ordinary cedar-wood oil is stated to have a refractive index ND 1.50567 at 17° which becomes ND 1.51682 when the oil is condensed.

New Multiple Staining Fluid.‡—Dr. P. G. Unna differentiates bacilli in tissues by a polychromic methylene blue solution which contains methylene red and violet, in addition to the blue. The sections are transferred from alcohol and allowed to remain in the stain for at least ten minutes. They are then passed through

* *Trans. Odont. Soc.*, xxv. (1893), pp. 176—177.

† *Phar. Jour.*, April 29th, 1893.

‡ *Phar. Journ.*, April 29th, 1893.

water into 33 per cent. tannic acid solution to decolorise, allowed to remain from two to five minutes, then rinsed with water to enable the exact tint to be observed more readily. If satisfactory, after a thorough washing with water, the sections are placed in absolute alcohol, or a solution of gold in the same if a yellow counter stain be desired, cleared in oil of bergamot, and mounted in balsam. If the excess of stain is not readily removed, a few minutes' immersion in 25 per cent. nitric acid, followed by dilute spirit, water, and absolute alcohol respectively, will effect its removal. By adopting this method it is said to be possible to distinguish two kinds of nuclei (violet and blue), the fibrine, and the protoplasm of the plasma-cells. The bacilli stain red, whilst the mucus surrounding them is blue, and the organisms are said to appear in their natural character, "in fish-roë like masses of vegetable mucus." It is claimed that the process is particularly suitable for use in the study of leprosy. It appears to depend upon the property, also utilised by Nicolle, by which tannin converts methylene blue into an insoluble form.—*Sheff. Med. Journ.*, L., 177.

Staining Reactions of Leucocytes.*—Wright and Bruce show that both "oxyphile" and "basophile" elements, according to Ehrlich's classification, attracting acid and basic dyes respectively, exist in leucocytes. The nucleus is invariably basophile, thus resembling cell nuclei generally, whilst the granules found in the leucocytes of the normal circulating blood in mammals are oxyphile. For complete histological differentiation, the two kinds of elements must be brought out in relief, and the following method of staining is suggested as satisfying the theoretical requirements. After fixing the cover-glass specimens of blood by dry heat or chemical reagents—such as osmic or picrid acid—the oxyphilous elements of the leucocytes are stained by floating the covers as long as may be necessary on a 1 per cent. aqueous solution of eosin or other acid aniline dye. The basophilous elements are next treated with a basic aniline dye, or with carmine or hæmatoxylin combined with alum to render them basic.

Leoeffler's methylene blue is recommended by the authors, on account of the rapidity of its action. Care must be taken to

* *Phar. Journal.*

control the various stages of the process by examining the specimens from time to time, as well as to check the action of the dyes at the right moment.—*B. M. J.*, No. 1,678, p. 400.

Method of Finishing Slides.*—The following is the process devised by Dr. Frank L. James (*St. Louis Med. and Surgical Journal*), and now in general use among American Microscopists: Precipitate an aqueous solution of gum arabic by adding alcohol until no further separation occurs. Filter to get rid of the liquid, and wash the precipitate on the filter with alcohol; collect, dry, and when dry redissolve the material in distilled water, to which about 1 per cent. of glycerin has been added. To this solution add about 2 per cent. of aluminium sulphate, and stir in sufficient talc, dry kaolin, or some such substance, to make a cement with a good body.

With this cement spin on your slips a ring of sufficient depth to act as a cell-wall for the mount. After mounting in balsam, or dammar, as soon as the surplus resin is dry, remove it with a knife or other means, and with a little benzol clean the slides around the cover-glass. Replace on the turn-table, and run round the edges a little of the cement, let dry, and then finish with Brunswick black, or any other of the oleagenous or resinous cements. The above method only applies to those preparations requiring a cell-wall. The ordinary balsam or dammar mounts, where no cell is necessary, are finished by removal of the surplus balsam, as directed above, and the application of the cement. For this class of mounts the addition of talc, etc., to the arabicin solution is not necessary, as the solution itself on drying leaves an intermediary coating impervious to benzol, chloroform, oil, or alcoholic solutions of the gums, etc., and Brunswick black applied to it will remain firmly adherent for an indefinite time. One precaution only is necessary, and that is to make the intermediary ring around the cover-glass so narrow that the finishing ring of Asphalt or Brunswick black shall overlap it both on the cover-glass and slide. Even a ring of plain gum arabic in aqueous solution will be sufficient to prevent the running in of the asphalte, etc.

* *National Druggist*, April, 1893.

Incoagulable Albumen (a new culture medium).*—M. E. Marchal has devised an albuminous solution, with which he has successfully cultivated a large number of bacteria, both pathogenic and saprophytic. The solution is prepared as follows :—The white of fresh eggs is diluted in distilled water and filtered. A solution of $1/1000$ of iron sulphate is then added to the albuminous liquid in the following quantities :—

Albuminous solution, 1—5 per cent., 1—5 centim. cubes per litre.

„ „ 5—10 „ 5—10 „ „ „

„ „ 10—15 „ 10—15 „ „ „

The iron sulphate has the curious property of preventing the coagulation of the albumen by heat. The liquids can be sterilised at once in an oven at 115° . The medium thus prepared is perfectly limpid, its reaction being slightly alkaline. M. Marchal finds that this solution is very easily and rapidly prepared, and it advantageously replaces the ordinary bouillons in use.

Half-an-hour at the Microscope, With (the late) Mr. Tuffen West, F.L.S., F.R.M.S., etc.

Callithamnion roseum (Pl. X., Figs. 1—8).—The origin of fruits by metamorphosis of branches into specialised structures of a higher type is beautifully shown. Two forms of fruit are seen. Special care must be taken to distinguish from fruits certain parasites, which are growths from spores of other attached algæ. Decurrent branches, adherent to the main stem, may also be seen with care. These have a peculiar interest, as shadowing out remarkable developments, in some other algæ, as *Batrachospermum*, and notably in species of a foreign genus, *Ballia*, in which they are developed to a remarkable extent. The medium employed in mounting (Aylward's fluid) appears to be a valuable addition to our materials for this purpose. I have seen Acari beautifully preserved in it, and should much like to know the formula for its preparation.

* *Bull. Soc. Belge Micr.*, XIX. (1893), pp. 64, 65.

Glandular Hairs of Sweet Briar (Pl. X., lower portion) are specially beautiful in the fresh state. Their interior is seen to be filled with soft, secreting, cellular tissue, and the tip to have a little projection of the fragrant essential oil, or "attar of roses"; this may be separated in a semi-crystalline condition by a little manipulation on the stage of the microscope.

Scalariform Ducts (Pl. XI., Figs. 1—6).—Different portions of these ducts vary greatly in the extent to which the regular ladder-like (*scala*, a ladder) structure is developed, as may readily be seen by examining the examples before us with high powers. Sometimes the latter deposits include two, or even three, of the earlier pores. Scalariform tissue is by no means confined exclusively to ferns. Good examples occur in the stems of Bryony, Vegetable Marrow, Cucumber, and the Eryngo or Sea Holly.

Sections of Fir (Pl. XII., Figs. 1—3).—To show the structure of wood in the Scotch fir, sections should be mounted in glycerine or chloride of calcium.

Disc of Ophiocoma (Pl. XI., lower portion).—I have not Forbes British star-fishes at hand, but believe this to be from *O. bellis*, the Daisy star-fish; the colour is natural and well preserved. I expect the white specimen alluded to by a member to be bleached. Different species of this genus vary much in size at maturity and the larger forms according to age. They cover from a mere point to a circle of seven inches in diameter or upwards, with a disc three-quarters of an inch across. The teeth of Echini furnish examples of, perhaps, the most wonderfully elaborate architecture to be met with in the whole animal kingdom. I have not specially examined the teeth of Ophiocoma, but am informed that they are simple, and make no approach to the complexity of those named.

Tongue of Trochus (Pl. XII., central portion).—I lately picked out several from a pint of "winkles" brought to the door for sale. Some species grow to a noble size; they are commonly known by the name of Top-shells.

In systematic description the teeth are said to be arranged in different series and described accordingly; thus, we have here

teeth of the median band single, wedge-shaped, with points slightly recurved; teeth of the lateral bands, five on each side, with strongly recurved points, the central the most so. The outermost bands are called "pleuræ," and having numerous teeth these are said to be indefinite. This arrangement is readily expressed by the formula, 00.5.1.5.00. As these parts are situated on the floor of the mouth, they cannot be correctly designated "palate," that being the roof of the mouth. Nor do I see any necessity for the new term "Odontophore" (tooth-bearer), the part being, in strict homology, the tongue, and fairly comparable to the tongue, with recurved points (cornified papillæ) of the ox or the cat.

Pediculus Capitis (Pl. XIII., Fig. 4).—The objects on this slide are small because they are youngsters, little more than just hatched out of the egg. They show, in an interesting way, the disproportion between limbs and body which we often note as giving a grotesque appearance to young animals of a higher type—calf, colt, puppy, or what not. Occasionally, examples may be met with just previously to moulting, where the soft parts are slightly withdrawn from the outer integument, previous to rupture of the latter, and exuviation. They then look like a louse within a louse.

Larva of Lace-wing Fly (Pl. XIII., Figs. 1-3).—The trumpet-shaped sucker (Fig. 2), terminating each limb between the claws, is only a special modification of a similar part so universally present in bees, ants, wasps, and the like, that it may be termed the "Hymenopterous" type of foot. Certain Neuroptera, as the Lace-wing fly, in its natural state, present good examples of a similar form.

Injected Human Lung (Pl. XII., Fig. 1).—In specimens such as these, it must always be remembered that we are viewing capillaries; minute vessels, intermediate in position between veins, carrying exhausted, and arteries bearing renovated blood. It may be doubted if any injections with two colours, as here seen, express correctly the anatomical facts of the case.

Selected Notes from the Note-Books of the Postal Microscopical Society.

Ophiocoma.—The disc of *Ophiocoma* is readily bleached by immersion in Liq. Pot., care being taken not to carry the process so far as to dissolve the integuments. A. NICHOLSON.

Larva of the Lace-Wing Fly (Pl. XIII., Figs. 1-3).—I send a drawing of this larva, concerning which I find the following particulars in "Westwood" :—The insect belongs to the order Neuroptera, family Hemerobiidæ, genus *Chrysopa*. It is distinguished, in its perfect state, by the brilliancy of its eyes and the delicacy of its wings. The larvæ feed upon Aphides, and are furnished with long curved mandibles, wherewith they seize and suck their prey. These mandibles, according to Westwood, are grooved beneath, and the maxillæ, which are of a similar construction, play in the groove. So ravenous are these insects, that it only takes half-a-minute for them to suck one of the largest aphides. They sometimes prey upon each other, the conqueror in like manner sucking the body of his victim. A. HAMMOND.

Section of Spinal Cord (Pl. XII., Fig. 2).—Quain's Anatomy gives figures of this, of which I copy a rough sketch and the following information :—The spinal cord consisted of white and grey nervous substance. The white matter (*cc*), forming by far the larger portion of the cord, is situated externally, whilst the grey matter (*dd*) is disposed in the interior. The principal fissures penetrate the substance in the middle lines (in addition to four lateral ones less marked), the anterior median fissure (*b*), and the posterior median fissure (*a*). The grey matter, separated by the shaded portions, presents two crescent-shaped masses (*dd*), joined across the middle by a transverse portion. Each of these crescents has an anterior, *ff*, and a posterior, *ee*, cornu or horn. A minute canal, represented by a very small circle, runs down the centre of the cord, and is lined with ciliated epithelium. A. HAMMOND.

Volvox globator, To Mount.—The gathering should be first strained through fine muslin and the residue placed on a glass slip while quite wet. Surround this with a ring of glycerine jelly, very slightly warmed, and finish off when cold with cement in the usual way. The secret of the bright green colour being preserved is to collect and mount at once, or as soon after collecting as possible. J. H. DAY.

Plates of Synaptia, to mount.—Some years ago I discovered that by soaking small pieces of the skin in Liq. Pot. for several

days, the skin became very clear and transparent. I laid them to dry upon the slide with another slide over them, and afterwards mounted in balsam. Though some of the anchors were broken, some on each piece of skin were quite perfect. By soaking longer the skin dissolves and the anchors and plates fall to the bottom. The sediment baffled me, but am told that if I had then used some rectified spirit the fatty matter would have been got rid of.

JOHN P. HALL.

Ditto.—Before placing to dry between glass slips, the softened skin of the synapta should have been put between pieces of paper. This would have prevented their sticking to the glass and subsequent breakage. A.

Spinal Cord.—This, as most persons know, extends from the brain to the lower part of the spine, and is the medium of communication between the nerves and the brain. It lies in a kind of tunnel, formed by the successive arches of the vertebræ placed in apposition to each other. I once met an advanced (!) student who did not know how the cord was situated. It is enclosed, as is the brain also, in three membranes. The innermost is excessively delicate, and is called *pia mater* (that is, "dutiful mother." This fanciful name, or, rather, its Arabian equivalent, was given it by the Arabians or other Eastern physicians, because of the persistency or affection with which it clings to the brain and cord). Next to the *pia mater* lies the middle membrane, called *Arachnoid* (that is, "Spider's web-like"). The outermost is called *Dura mater* (that is, "hard mother"). This is a stout and firm membrane, and probably escapes from the razor when one attempts to cut a section of it: at all events, it appears to be missing from the section before us. F. J. ALLEN.

Tentacle of *Physalia palagica* (Pl. XIII., Figs. 5—8), commonly known as the "Portuguese man-of-war." This specimen was given me by Captain Mortimer. The tentacles are from twenty to thirty feet in length, and consist of a muscular band, studded on its margin by a double row of beads, each bead being a mass of minute spherical cells, and each cell containing a spiral stinging-thread, coiled up cork-screw fashion, inside. Captain Mortimer, who is a distinguished naturalist, found the animal from which this was taken in the Pacific Ocean by surface-dredging. He had frequently witnessed the discharge of the stinging-threads from the cells, and stated that their stinging power was perceptible for some days after the death of the animal. J. C. THOMPSON.

EXPLANATION OF PLATES X., XI., XII., XIII.

PLATE X.

*Upper Portion.*Illustrations of *Callithamnion roseum*.

- Fig. 1.—The plant slightly magnified. $\times 10$.
 „ 2.—Branch more highly magnified, with favellæ (*f*), $\times 50$.
 „ 3.—Portion of main stem, with recurrent branches (*r. b.*, *r. b.*), $\times 100$.
 „ 4.—Favella, with tetraspore, $\times 200$.
 „ 5.—Spores in a “*ceramidium*,” or spore conceptacle, $\times 200$.
 „ 6.—Illustrates the origin of fruits by special modification of stunted branches, $\times 100$.

Lower Portion.

Illustrations of Glandular Hairs on Sweet Briar leaf.

- Fig. 1.—Portion of leaf showing upper surface, which is without glandular hairs, which are to be seen abundantly projecting round the edge.
 „ 2.—Lower surface, this is seen to possess scattered glandular hairs, $\times 5$.
 „ 3, 4, 5, are more enlarged figures taken from recent specimens; the soft internal secreting cells are seen highly coloured in the two first, and a little cap of Otto of Rose, on the tip of the hair, in figure 4, by manipulation, this has been somewhat dislodged, when it was under the microscope, exactly the well-known familiar semi-crystalline appearance of Otto. Almost colourless hairs, as in figure 5, are to be met with occasionally. Drawn by Tuffen West.

PLATE XI.

*Upper Portion.*Illustrations of Scalariform Ducts in Bracken (*Pteris aquilina*).

- Fig. 1.—Portion of bundle, $\times 20$.
 „ 2.—Large duct, with numerous elongated pores, $\times 250$.
 „ 3.—Duct, with shorter pores, on one part elongate, so as to include two and even three of the first pores, $\times 250$.
 „ 4.—Typical ladder-like, or Scalariform duct, $\times 250$.
 „ 5.—Is specially given to show the thickness of the walls, and projections of the “bars” in section, $\times 250$.
 „ 6.—Transverse view of bracken-stem, to show position of the ducts, which are surrounded and firmly embraced by thick walled pleurenychmatous tissue, $\times 2$.

Lower Portion.

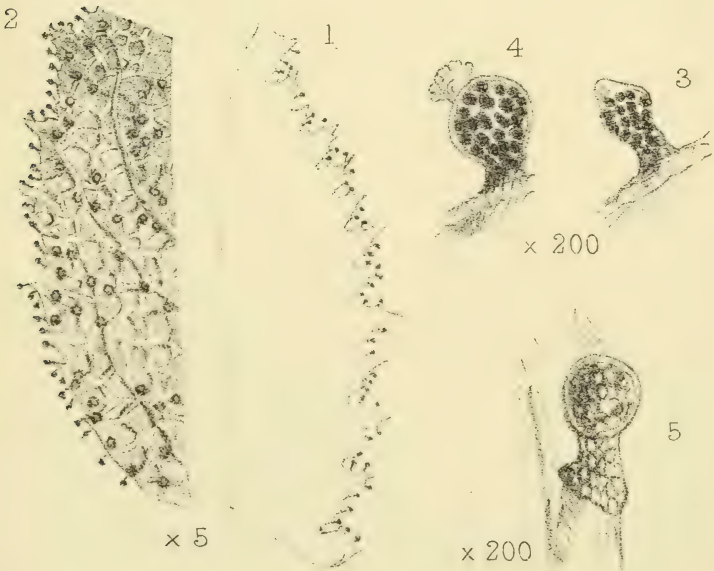
Illustrations of disc of Ophiocoma.

- Fig. 1.—View from above.
 „ 2.—View from beneath, showing mouth aperture, teeth, and plates, on which the latter work (“jaws”).

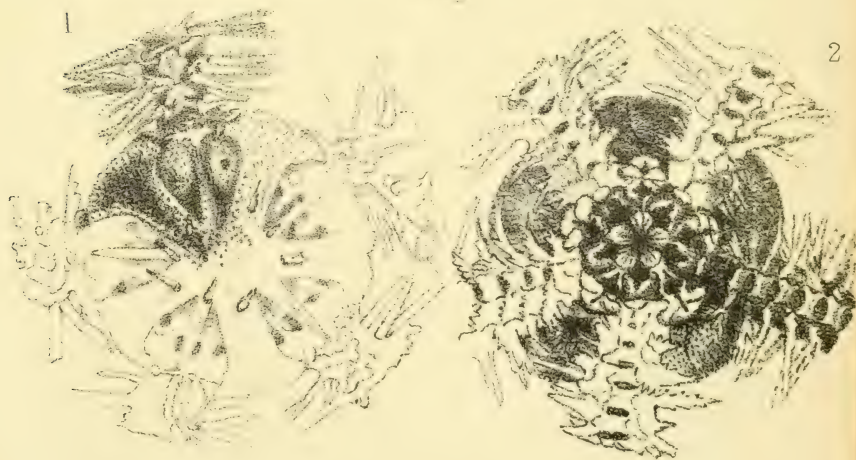
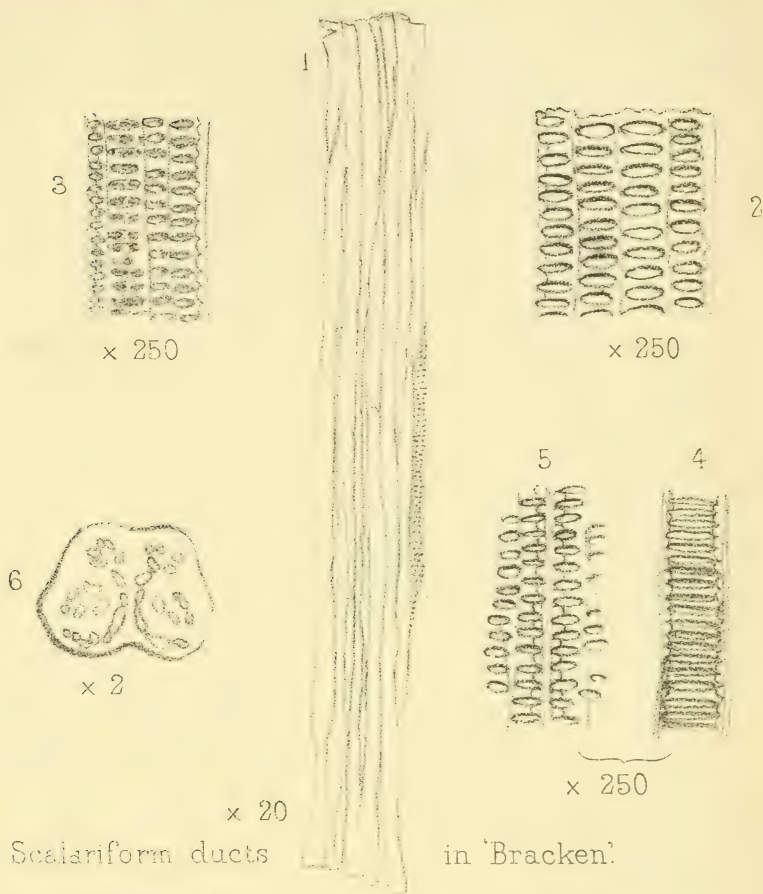
Drawn by Tuffen West.



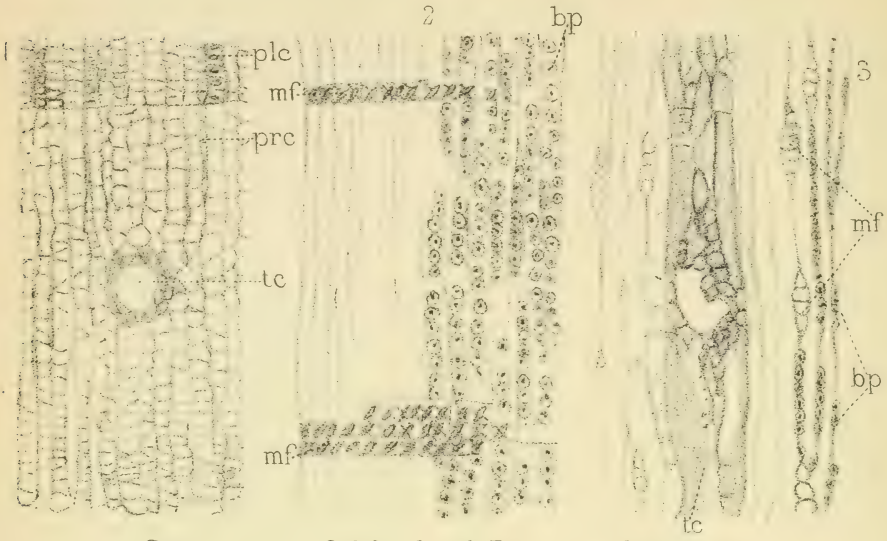
Callithamnion roseum.



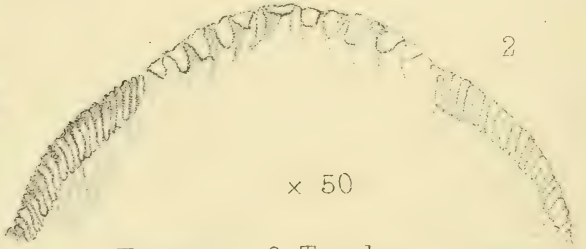
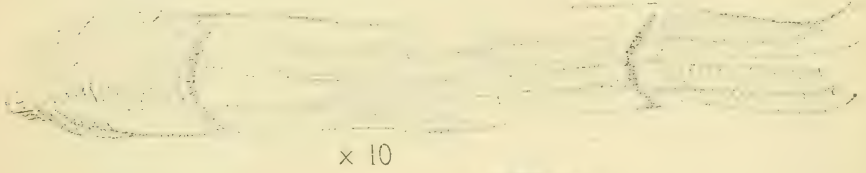
Glandular Hairs on Sweet-briar leaf.



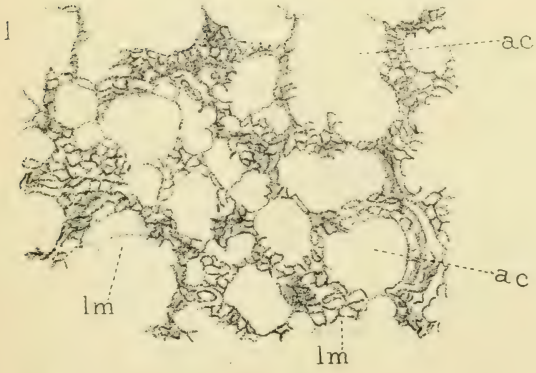
Disc of Ophiocoma.



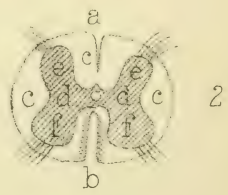
Structure of Wood of *Pinus sylvestris*.



Tongue of *Trochus crassus*.



Human Lung



Spinal Cord

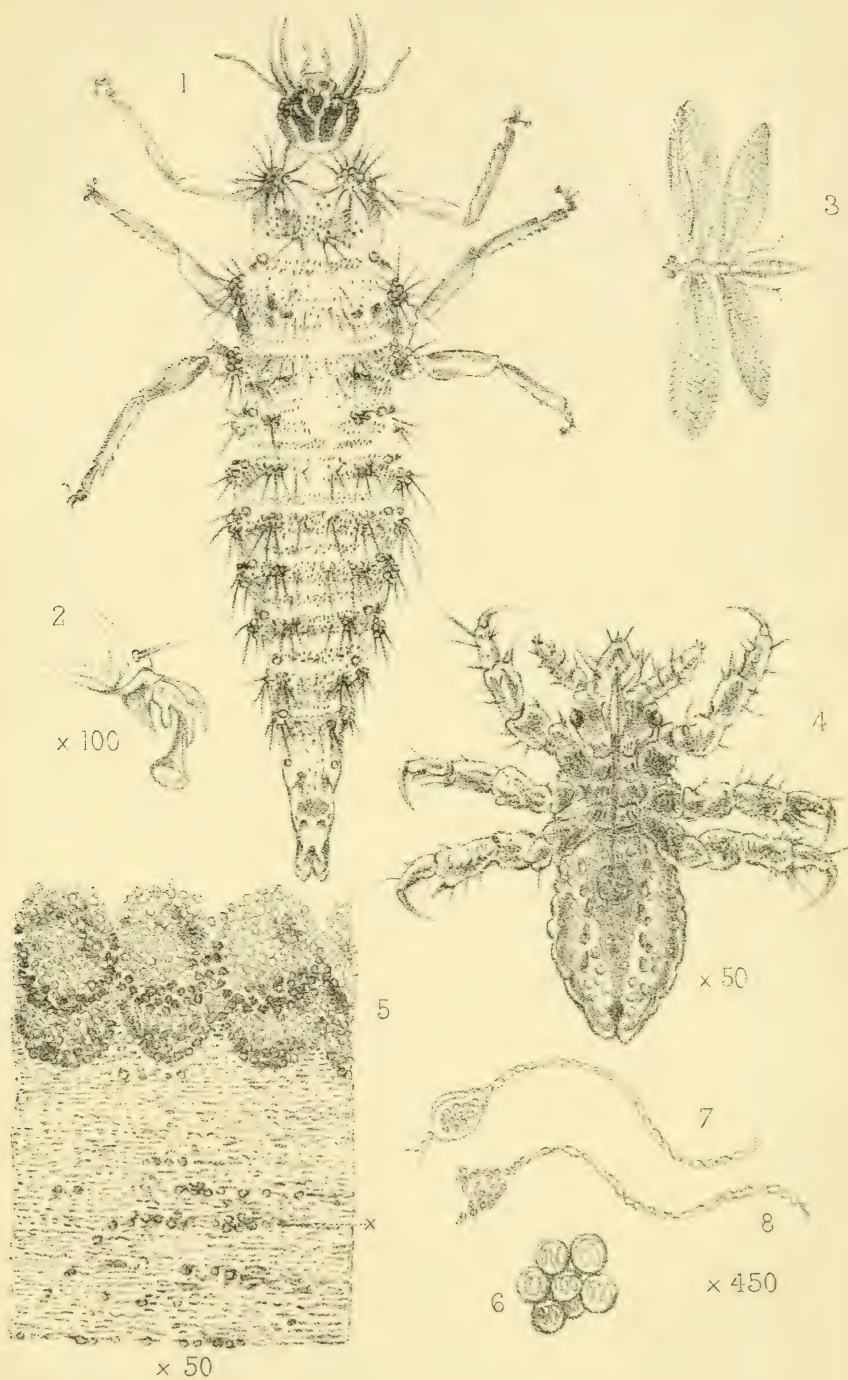


PLATE XII.

Upper Portion.

Illustrations of structure of wood of *Pinus Sylvestris*.

- Fig. 1.—Portion of a transverse section showing parts of the rings of growth; *plc.*, pleurenchyma, or woody fibre; *prs.*, pro-senchyma, or wood cells; *t. c.*, turpentine canal.
- „ 2.—Vertical radial section, with “bordered pits,” the so-called glandular tissue; *mf, mf*, portions of medullary rays.
- „ 3.—Tangential section, showing cross section of the medullary rays, and the bordered pits, as well as a large turpentine canal, *t. c.* All $\times 100$.

Central Portion.

- Fig. 1.—Tongue of *Trochus Crassus* entire, as viewed with a low power, $\times 10$.
- „ 2.—Teeth of a single series, $\times 50$.
- „ 3.—Shell of a species of *Trochus* to show its general character.

Lower Portion.

- Fig. 1.—Illustrating the structure of injected human lung, *a. c.*, *a. c.*, air cells; *l. m.*, *l. m.*, living membrane of air cells.
- „ 2.—Diagrammatic sketch of Spinal cord; *c c c*, white matter; *d d*, grey matter; *a*, the posterior; *b*, the anterior median fissure; *f f*, the anterior cornu or horn; *e e*, the posterior cornu.

Drawn by Tuffen West.

PLATE XIII.

- Fig. 1.—Larva of Lace Wing Fly, *Chrysopa perla*.

Drawn by A. Hammond.

- „ 2.—Diagram of left hind foot, $\times 100$.
- „ 3.—Lace Wing Fly, perfect insect from “Our Insect Allies.”
- „ 4.—Figure of young Head Louse, *Pediculus capitis*, $\times 50$.

Drawn by Tuffen West.

- „ 5.—Fragment of Tentacle, or muscular band of *Physalia pelagica*, $\times 50$.
- „ 6.—Spherical cells containing stinging threads coiled up inside, $\times 480$.
- „ 7.—Spiral thread, with cells containing granules of a blue colour, $\times 480$.
- „ 8.—Imperfect Spiral thread, with terminal of a brown colour, $\times 480$.

Many scattered cells will be seen in Muscular band also containing stinging threads.

Drawn by G. F. Chantrell.

Notes.

TWO kinds of corpuscles are stated to occur in the bacterial cell, one lying just inside the cell-wall and staining deeply with phenol-fuchsin solution; whilst the second is analogous to the nuclei of the higher vegetable cells. When unstained, the nuclei of the bacteria are said to resemble vacuoles.

—*Centr. f. Bakt. u. Parasit. Agric.*, vi., 384.

STRUCTURE OF THE BACTERIA.—Zettnow has published a paper in which he showed that the chromatin portion, usually seen when bacteria are stained by the ordinary methods, is the nucleus with its sheath. The plasma can only be seen when the specimen is treated with mordants. Leoffler's method was adopted in the experiments, and the preparations were afterwards photographed. *Spirillum repens*, *Proteus vulgaris*, *Chromatium okeni*, and the corkscrew bacillus were thus dealt with, and the plasma and nucleus were readily seen.

PROTECTION OF PLANTS AGAINST LARVÆ.—A. Laboulbène proposes to protect beetroot and cruciferous garden plants against the attacks of larvæ by the employment of decoctions of plants containing powerful alkaloids. Unlike mineral poisons—such as Scheele's green—the alkaloids lose their poisonous properties by undergoing oxidation upon the plants or in the soil, so that after performing the required work no risk of ulterior mischief need be anticipated in adopting this method. Repeated experiments have been made with decoctions of the stems and leaves of *Delphinium grandiflorum*, as well as with the seeds of the same plant, and of *D. Ajacis*. It is thought, however, that the seeds of *D. staphisagria* may be more energetic in their action, and the aconites, stramonium, belladonna, hyoscyamus, etc., are suggested as affording suitable material for experimenting in this direction.—*Compt. Rend.*, cxvi., 702.

FERTILISATION OF ORCHIDS.—J. H. A. Hincks, in discussing the fertilisation of orchids without pollen, quotes Professor Henslow, who shows how a microscopical examination of the structure of the essential organs at once renders apparent the reason of so small an amount of good seed being set. The pollen, instead of being in well-formed distinct grains, is arrested in development, and, while the grains are still in contact, a common extine clothes the whole of each mass. Development does not proceed until the pollen-mass has been placed upon the stigma. In the pistil, degeneracy is indicated by the prevailing parietal placentation and

by the rudimentary character of the ovules, every part of which is degraded. There is no albumen or nucellus-tissue to nourish the embryo, and the suspensor does its best to remedy this deficiency by elongating and escaping from the micropyle, then fastening itself like a parasite upon the placentas and extracting nourishment therefrom. As a result, myriads of seeds never succeed in developing even the pro-embryo.—*Science Gossip*.

COLOURING MATTER OF POLLEN.—G. Bertrand and G. Porrault claim to have established the identity of the colouring matter of yellow or orange pollens of diverse origin with carotin, $C_{26}H_{38}$, the substance to which the colour of carrots is due. From this generalisation they exclude the dry pollens found in the *Urticaceæ*, *Graminaceæ*, etc., which owe their yellow colour to the cutinisation of their external membrane. The abundance of fatty matters present prevented the crystallisation of the carotin of the pollen, but its iodide, $C_{26}H_{38}I_2$, was prepared. The coloured, crystal-like bodies that appear when pollen rich in oil is mounted in glycerine and examined microscopically, are not composed of carotin, but of a fatty body, probably cholesterin, with which the oil is supersaturated.—*Compt. Rend.*, cxv., 828.

Offered for Sale, a Private Collection of nearly 1,000 Slides, well-mounted and illustrating most branches of Microscopy; also a Collection of 236 Sea-weeds.—Apply to C. O. Sonntag, 171 Dalkeith Road, Edinburgh.

Reviews.

AN INTRODUCTION TO PRACTICAL BACTERIOLOGY for Physicians, Chemists, and Students. By Dr. W. Migula. Translated by M. Campbell and edited by H. J. Campbell, M.D., etc. Crown 8vo, pp. viii.—247. (London: Swan Sonnenschein and Co. 1893.) Price 6s.

Unless the student wishes to go deeply into the science of Bacteriology, the book before us will doubtless give all the information necessary. It goes minutely into the Examination of Living Bacteria, their structure, form, etc.; Preparation of Media, Cultivations, Staining, Mounting, etc. etc. There are two plates and nine illustrations in the text.

MODERN MICROSCOPY. I., The Microscope and Instructions for its Use, by M. J. Cross; II., Microscopic Objects: How Prepared and Mounted, by Martin J. Cole. 8vo, pp. 104. (London: Bailliere, Tindall, and Cox. 1893.) Price 2s. 6d.

The joint authors of the volume before us have given to the public a very useful book. The first section gives such advice as a novice requires before purchasing a microscope and its accessories, this portion of the book being well illustrated. The second section is by Mr. Martin J. Cole, whose mounted slides, and those of his fathers, Mr. A. C. Cole, are so well known and generally admired. Mr. Cole describes each separate stage of procedure in successive lessons or chapters, which if carefully studied and followed by the student,

must yield good results. We recommend this book to all beginners in microscopy.

PRACTICAL MICROSCOPY: A Course of Normal Histology for Students and Practitioners of Medicine. By Maurice N. Miller, M.D. Second edition. 8vo, pp. xv.—217. (New York: W. Wood and Co. 1893.)

We have much pleasure in noticing this book, which will doubtless prove very helpful to laboratory students. It is divided into three parts:—I., Technology; II., Structural Elements, as Cells, Connective Tissue, Cartilage, Bone, Blood-Vessels, etc.; and III., Organs, as the Skin, Teeth, Stomach, Lung, etc. There are 126 illustrations, which are photo-mechanical reproductions of the author's pen-drawings.

AIDS TO BIOLOGY. By Joseph W. Williams. 12mo, pp. 142. (London: Bailliere, Tindall, and Cox. 1893.) Price 2s.

This little book is specially prepared to meet the requirements of students reading for the first examination of the Conjoint Board, and aims to give in a condensed form the main facts of elementary biology. There are 39 very good illustrations.

THE ROMANCE OF ELECTRICITY. By John Munro. Cr. 8vo, pp. 320. (London: The Religious Tract Society. 1893.) Price 5s.

In a very entertaining manner, the author tells us about Electricity, which, "however practical, is perhaps the most romantic, as it is the most magical of all the physical forces." He treats of Thunder and Lightning, Fireballs, St. Elmo's Fire, Curiosities of the Telephone, Microphone, Electric Light, etc. The book is beautifully illustrated.

TEXT-BOOK OF COMPARATIVE GEOLOGY. By E. Kayser, Ph.D. Translated and edited by Philip Lake, M.A., F.G.S. 8vo, pp. xii.—426. (London: Swan Sonnenschein and Co. 1893.)

This translation of Dr. Kayser's "Lehrbuch der Geologischen Formationskunde" is intended to give to the English student a means of comparing the geological formations of various European countries. The geology of South Devonshire, for example, was only imperfectly understood until Mr. Ussher applied the knowledge which had been won in the Rhenish Mountains. The book contains 596 well executed illustrations (73 plates and 70 figures in the text) and a very full index.

BRITISH FUNGUS FLORA. By George Massee. In three vols. Vol. 2. Crown 8vo, pp. vii.—460. (London: Geo. Bell and Son. 1893.) Price 7s. 6d.

This classified text-book describes the genera belonging to the Ochrosporæ, Rhodosporæ, and Leucosporæ. On page 281 we have given one of the plates from this book.

THE GREAT SEA-SERPENT. By A. C. Oudemans. Roy. 8vo, pp. xv.—592. (London: Luzac and Co. 1892.) Price 25s.

This is an Historical and Critical Treatise, giving reports of 187 appearances, the suppositions and suggestions of non-scientific persons, and the author's conclusions. There are 82 good illustrations and a full bibliography. The author has gone to much trouble to make his work as complete and thorough as possible.

THE WILD RABBIT in a New Aspect. By J. Simpson. Cr. 8vo, pp. 135. (Edinburgh and London: W. Blackwood and Sons. 1893.) Price 5s.

The author has been practically interested in rabbit-farming on the estate of the Earl of Wharnccliffe, and shows how Rabbit-Warrens may be made to

pay. He treats of the demand for rabbits as an article of food ; the causes of failure of rabbit-warrens in the past ; the number of rabbits which one acre of grass will feed ; how to lay out a rabbit-warren, etc. etc.

LIBER VITE : Register and Martyrology of New Minster and Hyde Abbey, Winchester. Edited by Walter de Gray Birch, F.S.A., of the British Museum. 8vo, pp. xcvi.—335. (London: Simpkin and Co. Winchester: Warren and Son. 1892.)

A large amount of very painstaking research has been expended in the production of this book. **THE HYDE REGISTER**—the Stowe MS., No. 960—now in the British Museum, which has never before been committed to the press, illustrates the history of the Abbey in a variety of ways, many of which are as novel as they are instructive and entertaining. There are fac-simile pages of the MS. and several photographic plates from illuminated drawings. The frontispiece represents King Cnut and his Queen Ælfgifu bestowing a cross upon the altar of the Abbey, angels conducting saints, etc.

JARROLD'S ILLUSTRATED GUIDE TO CAMBRIDGE, LOWESTOFT AND SOUTHWOLD, HUNSTANTON, ALDEBURGH. Crown 8vo, pp. 143 ; 151 ; 92 ; 122. (London: Jarrold and Son. 1893.) Price 6d. each.

These four little guide-books—published by the enterprising firm of Jarrold and Son—are by far the nicest of the kind we have seen. They are well illustrated, and being of a size suitable for carrying in the pocket cannot fail to prove a welcome companion to the tourist. The guide to Hunstanton is written by Dr. J. E. Taylor, the well-known editor of *Science Gossip*, and, as might be expected, he gives a short description of the natural history of the district.

THE CONQUEST OF MEXICO AND PERU. By Kinahan Cornwallis. Crown 8vo, pp. vi.—443. (New York: *Daily Investigator* office. 1893.) Price \$1.00.

This is a well-written historical narrative-poem ; it is divided into four books:—1, The Discovery of the Pacific by Vasco Nunez de Balboa and the progress of discovery in the New World, from the first voyage of Columbus to the Conquest of Mexico ; 2, The Conquest of Mexico by Cortez ; 3, The Conquest of Peru by Pizarro ; 4, The Discovery of the Mississippi and the New World of To-Day. A thoroughly interesting book.

A NEW ENGLISH DICTIONARY on Historical Principles, founded mainly on materials collected by the Philological Society. Edited by James A. H. Murray, B.A. Lon., Hon. M.A. Oxon., LL.D. Edin., etc. etc. pp. viii.—861 to 1204. (Oxford and London: The Clarendon Press. 1893.) 12/6.

Part VII. of this very important work is now before us. Its contents includes the words from **CONSIGNIFICANT** to **CROUCHING**, and contains 5414 main words, explained in their alphabetical order, 936 combinations explained under the main words, and 1190 subordinate words, making a total of 7540. This part finishes the long series of words with the prefix *con-*, and contains also the by no means inconsiderable group of *contra-* and *counter-*. This is a book which we can take up and read by the hour together, finding fresh interest and instruction in every new paragraph.

ILLUSTRATIONS OF ELEMENTARY ANATOMICAL BOTANY. By Alexander Johnstone, Lecturer on Botany, Edinburgh School of Medicine. Sheet 1, The Root ; Sheet 2, The Stem ; Sheet 3, The Leaf ; Sheet 4, The Flower ; Sheet 5, The Flower ; Sheet 6, The Seed and Fruit. Size, 34in. by 28in. each. Price, Coloured, on Cloth and Rollers, Varnished, 3s. 6d. each, or 18s. for the complete series. (Edinburgh & London: W. & A. K. Johnston.)

The sheets of this series exhibit, in a simple, truthful manner, the general structural details of ordinary flowering plants. No. 1 gives a view of the Root System and its parts in different sections; No. 2, The Stem and Bud; No. 3, The Leaf and Leaf-Stalk; Nos. 4 and 5, First the Typical Flower as a whole, and then in parts; No. 6, The Fruit, Seed, and Embryo. The evolution of structures is indicated as occurring in nature, and the whole series can be taken as illustrating the elementary anatomy of a typical flowering plant. Several comparative sections are given through the series. At the foot of each sheet copious explanatory letterpress is given.

GRAPHIC ARITHMETIC AND STATICS. By John J. Prince. (London: Thomas Murby.)

This small manual is specially drawn up to meet the requirements of the Elementary and Advanced Stage of the South Kensington Science Examinations, and contains in a few pages much useful matter.

CONCRETE ARITHMETIC. By Temple Orme. Cr. 8vo, pp. 82. (London: O. Newmann & Co. 1892.) Price 2s.

This is an Introduction to the Elements of the abstract science of numbers for young children, in which instruction is given by the use of a number of wooden bricks, and certainly appears sufficiently simple for the youngest child to comprehend.

A HANDBOOK OF ALGEBRA. By Herbert Wills. Crown 8vo, pp. 264. (London: Jarrold and Sons.) Price 3s. 6d.

We have here set before the student, in a terse and methodic manner, the fundamental principles of Algebra. The greater part of the examples are taken from examination papers proposed to Pupil-Teachers, Scholarship, and First-year Certificate Candidates, and from papers set by the College of Preceptors, Oxford and Cambridge Locals, etc.

HOW TO SPELL and Speak English; with a Slight Sketch of the History of the Language. Second edition, revised by H. R. Ladell, M.A. 12mo, pp. 32. (London: Relfe Bros.)

The "Errors in Speaking" embody most of those set for correction in the examination papers of Oxford and Cambridge Locals, etc. Those in spelling consist of a list of 700 words in which mistakes are often made.

AN INTRODUCTION TO SCIENTIFIC CHEMISTRY. By F. S. Barff, M.A. Crown 8vo, pp. xvi.—200. (London: O. Newmann and Co. 1893.) Price 4s.

This is a new edition, revised by T. Orme, Teacher of Chemistry in University Coll. School, London, etc. He uses throughout the book the systematic nomenclature of Dr. A. W. Williamson, and in a small space conveys a good deal of useful information.

THE SURGICAL ANATOMY AND SURGERY OF THE EAR. By Albert H. Tuttle, M.D. Fcap. 4to, pp. vii.—109.

APPENDICITIS AND PERITYPHLITIS. By Chas. Talamon, M.D. Fcap. 4to, pp. vi.—210. (Detroit, Mich., U.S.A.: Geo. S. Davis. 1893.)

These volumes treat their respective subjects in a very thorough manner. The one on the ear is illustrated with 22 fine photo-mechanical plates, in which the anatomy of that organ is fully explained.

MOTHER AND CHILD. Part I., Mother, by Edward P. Davis, A.M., M.D. Part 2, Child, by J. M. Keating, M.D., LL.D. 8vo, pp. 472. (Philadelphia: J. L. Lippincott and Co. London: 10 Henrietta St., Covent Garden. 1893.)

The object of the authors of this book has been, not to supplant the physician, but to supplement his advice, and render intelligible those matters that mothers and nurses find difficult to understand. There are several illustrations.

CRIMINOLOGY, by Arthur MacDonald; with an Introduction by Dr. Cesare Lombroso. Crown 8vo, pp. 416. (New York: Funk and Wagnalls. 1893.) Price 82.

The science of Crime and Criminals is treated in a very thorough and scholarly manner by the author, as the result of years of expert study and research, and the work closes with some practical conclusions which deserve careful attention. At the end of the book is an exhaustive biography of crime, occupying some 120 pages.

INDIGESTION Clearly Explained, Treated, and Dieted. By Thomas Dutton, M.D. Univ. Durh. Second edition. Cr. 8vo, pp. iv.—143. (London: Henry Kimpton. 1893.) Price 2s.

This book will be read with pleasure and, we hope, profit by those who suffer from indigestion. The instructions are given in plain language, and may, we believe, be followed with great advantage to the sufferer. The book treats also of Gout, Constipation, and Obesity, and has a chapter on the Rearing of Infants.

MR. H. K. LEWIS, 136 Gower Street, London, has sent us some cards:—**DISINFECTANTS AND ANTISEPTICS**: How to use them. By E. T. Wilson, M.B., L.R.C.P.

One at least of these cards should be found in every house. They are sold at 1s. per dozen or 1/1 post free.

PRACTICAL POCKET-BOOK OF PHOTOGRAPHY. By Dr. E. Vogel. Translated by E. C. Conrad, F.C.S. Crown 8vo, pp. x.—202. (London: Swan Sonnenschein and Co. 1893.) Price 2s. 6d.

The author gives, in small compass, an account of most of the important photographic processes. The formulas given are good, and have been practically tested, and are limited to those in use in the Photo-Chemical Laboratory of the Royal Technical High School. There are upwards of 60 illustrations.

THE AMATEUR PHOTOGRAPHER'S ANNUAL, 1893. 8vo, pp. xix.—216. (London: Hazell, Watson, and Viney. 1893.) Price 2s. net.

The Annual is well illustrated. It contains one carbon print, thirteen colotypes, and a number of process-block illustrations. We find in it a complete guide to carbon-printing, a number of practical articles (in which are some valuable hints), and a holiday guide to the leading photographic haunts in the empire. It is a cheap book.

ELEMENTARY PHOTOGRAPHY. By John A. Hodges. Cr. 8vo, pp. 159. (London: Hazell, Watson, and Viney. 1893.) Price 1s.

In the (No. 7) Amateur Photographer's Library the author explains, in simple language, the various processes connected with the production of a photograph, from the buying of the necessary apparatus to the mounting and finishing of the print. There are several illustrations.

GOD'S BIRDS. By John Priestman. Crown 4to, pp. 91. (London : Burns and Oats. 1893.) Price 3s. 6d.

A handsomely got-up volume, in which we find some very interesting anecdotes and facts about the birds mentioned in the Bible. The book is written in language quite adapted to the understanding of young people.

PHILLIPS BROOKS' ADDRESSES, with Introduction by Rev. Julius H. Ward. Crown 8vo, pp. 176. (Boston : Charles E. Brown and Co.)

It is said of the late Bishop Brooks that he had the rare faculty of never speaking nonsense. The volume before us contains six addresses on various subjects, the last being on Abraham Lincoln.

SOCIAL LIFE AMONG THE ASSYRIANS AND BABYLONIANS. By A. H. Sace, LL.D. Crown 8vo, pp. 127. (London : The Religious Tract Society. 1893.) Price 2s. 6d.

This little book, one of the "By-paths of Bible Knowledge" series, describes in an interesting manner the People and how they lived, their Education, etc., the Market, the Money-Lender, and the Tenant, etc.

HELPS TO THE STUDY OF THE BIBLE. Crown 8vo. (Oxford and London : The University Press.)

Sunday-School Teachers and Students will find this a most useful work. It consists of upwards of 700 pages, and comprises compendious and exhaustive information on all points of Biblical Study—Analytical, Critical, Chronological, Historical, and Geographical, a Glossary of Antiquities, Dictionary of Proper Names and Subjects, a Concordance, and a new Indexed Atlas, with 15 maps and 64 full-page plates.

HEBREW IDOLATRY AND SUPERSTITION : Its Place in Folk-Lore. By E. Higgins. Crown 8vo, pp. x.—80. (London : Elliot Stock. 1893.) Price 3s. 6d.

The author argues that the idolatrous customs of the Hebrews were vestiges of the religion of former inhabitants of the countries. The various chapters treat of Traditional Religion ; The Religion of the Soil ; Amorite Religion and Worship of Heavenly Bodies ; and Divination, Witchcraft Enchantment.

THE LEGENDARY LORE of the Holy Wells of England. By Robert C. Hope, F.S.A., F.R.S.L., etc. 8vo, pp. xxx.—222. (London : Elliot Stock. 1893.) Price 7s. 6d.

We have here accounts of all the Holy Wells of England, including celebrated Rivers, Lakes, Fountains, and Springs. They are arranged according to the counties in which they are situated. We judge from the index that some 600 Holy Wells are mentioned. There are some curious illustrations.

CHESS : HISTORY AND REMINISCENCES. By H. E. Bird. Crown 8vo, pp. xxiv.—138. (London : Dean and Son, Limited.)

Lovers of Chess will find in this work plenty of good reading bearing on such points of interest as Styles and Oddities of Chess-Players ; Origin of Chess : Its Progress in the Early and Middle Ages ; and, of course, chapters on the "Blindfold" game, in which the author has achieved such success. Mr. Bird has condensed within these pages much information, gleaned from his past experience and intercourse with the leading players of the age.

CHEIRO'S BOOK OF THE HAND. Crown 8vo, pp. 100.

PALMISTRY MADE EASY ; or, Hand-Reading for Beginners. By M. J. Chapman. Crown 8vo, pp. 58. (London : The Record Press.)

Those interested in this subject will doubtless be pleased to read these little books. They are published at 1s. 6d. each.

The Spongida or Porifera.

BY R. LAWTON ROBERTS, M.D., AND MISS FLORENCE PHILLIPS.

PLATES XIV., XV., AND XVI.



SINCE the time of Aristotle, the sponges have been regarded as interesting objects of enquiry, but during the present century they have proved a fertile source of discussion, of warm controversy, and of laborious investigation on the part of skilled biologists.

Formerly, the point at issue was whether the Spongida were of the nature of plants or animals, or whether or not they constituted a connecting link between the vegetable and animal kingdoms. Latterly, since it was shown by Grant that Sponges possessed animal characteristics, the greatest differences of opinion have arisen as to the exact position occupied by them in the scale of animal life.

Many competent observers insist that the Sponges are closely allied to the PROTOZOA, minute and lowly animal forms, composed of homogeneous or somewhat granular, gelatinous substance or sarcode, devoid of any true organs or any proper cellular tissue, and in some instances possessing cilia, flagella, or tentacula. As typical of this side of the question, the view expressed by H. James Clark in 1868 may be mentioned, that the Sponges "must be regarded as compound colonial forms" of Flagellate Infusoria.

Quite an opposite theory was advanced, in 1869, with great ingenuity and forcible argument, by Ernst Haeckel. This authority urged that the association of the Sponges with the Protozoa was an error; that, on the contrary, the Sponges were of a higher grade, being properly allied to the Corals and Zoophytes, or CÆLENTERATA. He insisted strongly that the canals which permeate the Spongida, were comparable with the digestive-circulatory cavities of the Corals, that the spaces, immediately including the larger orifices (or "oscular area") of a sponge, represented individual Polypes, and that the reproduction of the Sponges was effected, as in the Corals, by means of ciliated larvæ (or "*gastrulæ*"),

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these being formed from the ordinary development and segmentation of ova. In 1872, Haeckel published his Monograph of the *Calcispongiae*, in which he still further insisted on the near connection of the Sponges with the Corals, declared that he had observed spermatozooids, as well as ova, in several species of the Spongida, and that the ciliated larva or gastrula, from which all Sponges developed (and which occurs throughout the animal scale from the Sponges up to the Vertebrates, "as represented by *Amphioxus*"), was an embryonic representation of an ancient and primitive animal form termed "*Gastræa*," from which the various animal types have descended.

The eminent position of Haeckel, and the ingenuity, the force, and brilliancy of his writings and arguments, took—so to speak—the biological world by storm; and his teaching became widely accepted, his opinions quoted, and his illustrations copied by the authors of many subsequent standard works and text books. All this, however, caused renewed and increased attention to be directed to the Spongida, which, in consequence, have been most perseveringly studied by numerous biologists, with the purpose of verifying or refuting the views emphasised by Haeckel. Indeed, so much has been written on the subject, that we are reminded of the dictum laid down frequently and emphatically by the eminent surgeon—the late Mr. Richard Quain:—"When you find that a great deal has been written on any subject, you may feel quite sure that very little is known about it!" This may seem to many a hard saying, yet it is not inapplicable to the present case. There is still great difference of opinion as regards the true position of the Sponges in the animal scale. "English Biologists," the writers are informed, "as a rule, prefer to place them in a group by themselves—the PORIFERA; while continental men—Haeckel and Lang among them—place them under the Cœlenterata." Others there are again, who still consider the true position of the Sponges to be with the PROTOZOA. Saville Kent, for example, in his voluminous and learned "*Manual of the Infusoria*," 1881, argues with great force and ability that the *Spongida* are very closely connected with the flagellate collared Infusoria; and in the system of classification adopted by him, the independent flagellate collared monads form Section I., and the Sponges Section II., of his Order, *Choano-Flagellata*.

There can be no doubt that the wide differences of opinion regarding the "nature and affinities of the Sponges," result, in a great degree, from the difficulty attending accurate and continued observations and study of minute *living* forms, such as the Infusoria, and the Flagellate and other cells which play so important a part in the life-history of the Sponges. It will be necessary to allude later on to this matter, and also to some of the arguments adduced by Saville Kent. At present we feel disposed to suggest that some of those interested in Biology, who have difficulty in determining the particular group of living forms on which to devote special attention, may—with advantage to themselves, and possibly to science—select the *Spongida* as a speciality. There can be no lack of material to work upon. There is the fresh-water *Spongilla*, and numerous marine species are found in abundance on the British coasts. In these days of the parcel post, living specimens can be easily and successfully transmitted in sea-water from seaside resorts to inland counties. We have often received Sponges, alive and vigorous, from the Channel Islands by means of the parcel post. This is a highly important matter, for it is not sufficient to examine only the general conformation, the spicules, the canal system, the dried or preserved fabrics of different species of the Spongida. Every effort should be made to obtain as many species *living* as possible, so that the vital elements of the Sponge, the flagellate collared cells, the amœmibiform cells, the "ciliated larva," or gastrula, may be studied as minutely as possible.

Considerable practical experience in working with the microscope, the preparation of slides, and the use of very powerful objectives, will be required in the pursuit of such a study. Saville Kent, when investigating the Infusoria, adopted means of pursuing his researches which those who propose to study minute living forms would do well to imitate. Instead of the usual "cover-glass," *talc*—such as is used for glass shades—is recommended. This may be readily split into scales of exceeding lightness, transparency, evenness, and thinness; and such scales can be easily cut with scissors, and bend so easily that objectives may be racked close down on the objects to be examined. It is stated that with such thin talc covers, the use of 1/16th, 1/25th, and 1/50th inch objectives becomes comparatively an easy matter.

Then again, when it is required to study minute living aquatic forms, there is considerable difficulty in keeping the slide on the microscope stage sufficiently moist. There is a capital paper by Messrs. Dallinger and Drysdale in the *Monthly Microscopical Journal*, March, 1874, accompanied by illustrations, showing their "method of preventing the drop of fluid under examination from evaporating, so as to admit of continuous examination of the same forms with the highest powers." This plan is re-told, in an exceedingly clear style, by Saville Kent, as follows:—"It consists firstly of a plain glass stage, about the one-tenth of an inch thick, fitted so as to slide on in place of the ordinary sliding-stage of the microscope. From the left hand anterior border of this stage a projecting arm is produced, which carries a socket for the reception of a small glass reservoir, about one-and-a-half or two inches deep. The glass stage being too thick to work through with an achromatic condenser and high powers, a circular aperture of sufficient size is cut through it, and a piece of thin glass cemented on its upper surface. A piece of blotting-paper is now cut coinciding in form with the glass stage, but slightly smaller, and with a tongue-like projection that lies along the projecting arm, and dips down into the glass reservoir. A circular aperture of larger size than the covering-glass employed is cut out of the centre of this paper, such aperture, where a $\frac{1}{4}$ -inch cover is made use of, being preferentially the $11/16$ ths of an inch. The foundation of the moist chamber is now complete, and it only remains to provide the bounding walls. This Messrs. Dallinger and Drysdale accomplish by means of a piece of glass tubing, about one-and-a-half inch in diameter, cut to three quarters of an inch in length. Across one end of this tubing a thin sheet of caoutchouc is next firmly stretched and securely tied, and a small hole perforated in its centre. The tubing with its free edge, which should be carefully ground, is now placed concentrically upon the glass stage, over the aperture in the blotting paper, and the object-glass racked down upon the perforation in the caoutchouc. The caoutchouc should be sufficiently thin to offer no impediment to the action of the fine adjustment, while it at the same time clasps the object-glass firmly round its central perforation and in combination with the lowermost, or free edge, resting on the

blotting-paper, constitutes a practically air-tight chamber. Everything is now in working action, and it only remains to add the material to be examined, and to fill the reservoir with water. The water from the reservoir soaking through the bibulous paper, keeps the air-tight chamber constantly moist, and evaporating faster from its contained free circular edge, prevents loss of moisture from beneath the covering-glass. The water in the reservoir will maintain the moist chamber in the above conditions for many days, and will require replenishing only at distant intervals." If continuous observation is not requisite, and the microscope is needed for other purposes, the slides may be transferred to an "ordinary moist chamber, which may be extemporised out of a tumbler, or small bell glass, inverted upon a plate containing a few folds of well-saturated bibulous paper."

For "obtaining the most satisfactory illuminations and definition of minute flagellate organisms," Saville Kent adopted a plan recommended by E. M. Nelson, F.R.M.S. This necessitates a horizontal position for the microscope. "The mirror being turned to one side, the microscope and lamp are so disposed that the central ray of light from the *narrow edge* of the lamp flame passes through the optical axis of the condenser, and is then focussed upon the field of view, by means of the substage rackwork, in such a manner that, employing a 1-inch object glass, a sharply defined image of the lamp flame, edge on, is projected upon the centre of the field in company with the objects under examination. If the 1-inch object glass is now detached, and a 1/16th, 1/25th, or 1/50th substituted, and focussed into place, a slight re-adjustment of the centering of the achromatic condenser being perhaps required, it will be found that the entire field is brilliantly illuminated, and the most minute objects defined with an amount of sharpness rarely obtained under other conditions. In addition to the ordinary graduating diaphragm, "the interposition of a second diaphragm at the lowest point in the substage arrangement" is of considerable advantage.

We have quoted this method, as Saville Kent found it so useful in examining minute infusorial forms with high-power objectives ranging from 1/16th to 1/50th. Good work can be done, however, by means of a 1 12th inch object glass with an Abbé's condenser.

With this arrangement we have seen easily (working by day-light) the flagella, collars, neuclei, and neucleoli of the collared cells of *Sycandra ciliata*.

We have before us five specimens of British Spongida (Plate XIV.), all of which are interesting to us, some from personal associations, others as representing species of which noteworthy observations were made by distinguished biologists who have passed away, as Grant and Bowerbank, etc. The two specimens (Figs. 1 and 2, Plate XIV.) of the Crumb-of-Bread Sponge (*Hatichondria panicea*) are very different in general form on account of the different points of attachment from which they grew. The first specimen (Fig. 1) was scraped off a rock, and is of a flat shape, except for the projecting conical or teat-like eminences, fourteen in number, which jut out abruptly, some to the extent of half-an-inch, from the flat roughly-porous sponge mass. At the free end of each of these projections is a round or oval orifice (*a, a, a*)—the termination of a canal leading from the sponge. These apertures averaged about one eighth of an inch in diameter, some being larger, others rather less, and are termed the *oscula* or *fecal orifices* of the Sponge. The Sponge as a whole is of a light grey-brown tint, of an exceedingly open and porous texture, and measures about one and three-quarter inches across, from two to three inches in height (one border being shorter than the other), yet only averages a quarter of an inch in thickness between the conical prominences. The entire thickness of the Sponge, if we include the largest cone-like projections (that is, measuring through the Sponge from its base of attachment to the rock to the most prominent osculum), does not exceed three-quarters of an inch. The hinder surface of the Sponge, which has been torn off the rock, is flat and porous, with many fragments of acorn shells embedded in its substance.

Our other specimens of *H. panicea* (Fig. 2, Plate XIV.) presents a very different appearance, and it is of interest to us since it was the first living sponge we witnessed in a state of activity, and discharging, for several hours continuously, strong currents from the oscular orifices. The following observations were made regarding this identical specimen in another paper (published in the *Field Club*, July, 1892):—

"We received one morning, by parcel post, in a bottle containing nearly a pint of sea-water, a living specimen of the common crumb-of-bread sponge (*Halichondria panicea*). Roughly speaking, our specimen consisted of a yellowish-grey, porous, spongy-looking, irregularly shaped mass, enveloping, and attached to a very narrow-branched and black piece of sea-weed; the irregular form of the sponge being chiefly due to teat-like projections from the general surface, each of these conical elevations having a large hole or orifice at the summit. To particularise, the following are our detailed notes:—

"The sponge is slightly more than two-and-a-half inches in height, and varies in breadth from two-thirds of an inch to one and two-thirds of an inch, and in depth (that is, from before back) from two-thirds of an inch to one and a quarter inch. The shape is peculiar and very irregular, the upper third of the mass being roughly globular, and separated by a slight constriction from the lower part, which increases in girth and breadth to nearly the base, where there is a transverse split one and a quarter inch long, revealing an empty sulcus, or channel (*b* 1), which has evidently been once filled by the narrow frond of a sea-weed. The entire sponge, indeed, completely envelops, and has grown upon, a branched piece of sea-weed; the dark-coloured broken edge of the latter (not a quarter of an inch in diameter) crops out about the middle of the sponge (*b*), a similar fragment is apparent near the base, and through the substance of the upper part of the mass more can be discerned.

"The fore-part of the sponge, from the transverse fissure at the base to the top, is remarkably irregular, this irregularity being due to nine more or less prominent, conical, or teat-like projections, roughly arranged in two vertical rows. The most prominent of these is one third of an inch in diameter, and projects at least half-an-inch from the body of the sponge, and the others stand out in varying degrees; but all have a large oval or rounded orifice (*a*, *a*) or *osculum* (one-twelfth of an inch or more in diameter) in the centre, down which can be easily seen a channel running towards the body of the sponge. The hinder portion of the sponge is much smoother, no prominences or large openings being apparent; and this surface, too, is of a greenish tint,

whereas the general hue of the entire mass is yellowish-grey, and its appearance porous and 'spongy.'

"We placed the glass jar, having first removed its covering, on the window-sash, so as to have the sponge between ourselves and the sunlight; and at once had the satisfaction of seeing that our specimen, in spite of two days' knocking about by parcel post, was not only living, but in a condition of wondrous activity. The glass was so placed that we had a side view of the teat-like projections, and issuing from the orifices of the latter we could readily see, without the aid of a magnifying glass, a continual and rapid rush of numberless particles; these issued in a horizontal column from each cone, and gradually broke up into clouds of minute specks about an inch from the sponge.

"The sight immediately reminded us of the popular pictures of volcanoes in a state of eruption. If a number of such drawings were reproduced in miniature, and placed one above the other, horizontally, and pointing all the same way, a very fair idea would be given of our *Halichondria panicea* at work.

"It is of great interest to note Dr. Grant's original observations as to this matter, since he it was who first cleared up the true nature of the Sponges. He was examining another species—*Spongia coalita*—and witnessed the same spectacle that we have attempted to describe.

"He says:—'On moving the watch-glass, so as to bring one of the apertures on the side of the sponge fully into view, I beheld for the first time the splendid spectacle of this living fountain vomiting forth from a circular cavity an impetuous torrent of liquid matter, and hurling along, in rapid succession, opaque masses, which it strewed everywhere around. The beauty and novelty of such a scene in the animal kingdom long arrested my attention, but after twenty-five minutes of constant observation I was obliged to withdraw my eye from fatigue, without having seen the torrent for one instant change its direction, or diminish in the slightest degree the rapidity of its course. I continued to watch the same orifice, at short intervals, for five hours, sometimes observing it for a quarter of an hour at a time, but still the stream rolled on with a constant and equal velocity.'

"The sight which Dr. Grant described so vividly we witnessed

in a greater degree while observing our *H. panicea*, for we saw not one, but nine 'living fountains vomiting forth' from the large apertures (or *oscula*, as these are technically called) of our sponge, and this kept on for several hours without intermission."

Our third specimen (Fig. 3, Plate XIV.) is a pear-shaped Sponge, two and a quarter inches in length, one and a half inches across at the broadest part, and a little over an inch thick at the large end. It is of a dull grey hue, firm to the touch, a little worm-eaten in parts, and has grown upon and enveloped two branched pieces of sea-weed (*b, b*). The weed in this instance is *Chondrus*; the fragment associated with the specimen previously described is *Fucus*. Scattered here and there about the sponge, mostly on the side depicted in the illustration, are variously sized oscula (*a, a, a*) the largest being three-tenths of an inch in diameter, not raised on conical or mammæ-form projections as in our two previous specimens, but level with the surface of the body of the sponge, and looking like holes punched in the latter by a miniature cork-borer. This Sponge is of considerable interest to us, since it is the *Halichondria encrustans* of Johnston, and the *Spongia panicea* of Grant. "The *Spongia panicea*," wrote Grant in 1825, "presents the strongest current which I have seen. . . Two entire round portions of this sponge were placed together in a glass of sea-water, with their orifices opposite to each other, at the distance of two inches: they appeared to the naked eye like two living batteries, and soon covered each other with feculent matter. I placed one of them in a shallow vessel, and just covered its surface and highest orifice with water. On strewing some powdered chalk upon the surface of the water, the currents were visible at a great distance, and on placing some small pieces of cork or of dry paper over the apertures, could perceive them moving, by the force of the currents, at the distance of ten feet from the table on which the specimen rested. A portion of soft bread, pressed between the fingers into a globular form, with a diameter larger than that of the orifice, and placed over it, was not moved away in a mass by the stream, but was gradually worn down by the current beating on its sides, and thus propelled to a distance in small flakes. A portion of unburnt black coal, with twice the diameter of the orifice, was instantly rolled off the mouth of this living fountain, in whatever position I attempted to make it rest upon it."

During continued and repeated observations of various species of Sponge, Grant noticed that the currents invariably flowed in the same direction through the oscula (*a, a, a, a, a*, Figs. 1, 2, 3, Plate XIV.), viz., from the interior of the sponge outwards. For the purpose of avoiding all chance of error, and thinking it possible that, during the close observation of a single osculum extending over a lengthened period, the currents through other oscula of the same sponge might be reversed and flowing towards the interior, he "took from the rocks some specimens of the *Spongia compressa* (Fig. 4, Plate XIV.), constructed like a small white paper bag, with only one round aperture at the extremity of the body; and on placing each in succession under the microscope with sea-water, found that each of these animals sent forth from the only aperture of their bodies a slow but constant stream."

Our specimens of this Sponge (Fig. 4, Plate XIV.), the *Spongia compressa* of Grant, but better known now as *Sycandra compressa*, resemble creamy white compressed or flattened bags, or sacks, slightly stalked at the base (where previously attached to sea-weed, or less commonly to rocks), oval or ovate in form, usually broader towards the base, and narrowing gradually up to the single rounded osculum (*a*), which measures from a half-inch to one-third of an inch in diameter. Our specimens measure about one inch across at the broadest part, and from one and a quarter inches to two inches in length. The large specimen is a little worm eaten. More oscula than one are formed in old growths, but these natural openings occur at the angles or margins. It is noticeable that, in the largest sponge of the group, the lateral dimensions have not increased correspondingly with the length.

After numerous observations, having noticed that the water currents invariably passed from the interior of the sponge through the oscula or larger openings outwards, conveying quantities of excrementitious matter, Grant suggested that these openings should be termed *fecal orifices*, in contradistinction to the minute apertures or *pores*, scattered all over the surface, through which the water passed into the sponge.

The same observer pointed out that the different position of the *fecal orifices*, or *oscula*, in various specimens depended greatly on the direction and manner in which the Sponges grew. Thus

he said :—"The fæcal apertures are raised to the extremities of projecting papillæ, in such sponges as cover the sides of rocks, in order to convey the excrement beyond the pores and general surface of the animal. In such branched species as have a soft, downy surface, the fæcal orifices are ranged in close order along the outer margins of the branches, and very few are observed on the flat surface, in order to prevent the excrement from falling in the direction of the flat woolly surfaces, which would be very apt to retain it, and thus choke up the groups of pores which are seen everywhere over their surface. Such branched sponges have not, and do not require, projecting papillæ, because they hang suspended by a narrow stem, and are kept sufficiently clean by receiving gentle undulations from the constant motions of the sea. The same applies to the soft, downy, white *Spongia compressa* (Fig. 4, Plate XIV.), which always hangs down and whose orifices are always marginal. The bright yellow, porous, placentiform mass of the *Spongia panicea* (*H. encrustans*, Fig. 3, Plate XIV.) has no papillæ; indeed, the fæcal orifices are sometimes even lower than the general surface of the animal, and I have never seen this sponge, excepting on the under surface of rocks, with its orifices perpendicularly downwards, so that the excrements fall clear of its surface by their own gravity, without the assistance of papillæ. The flat species which are found encrusting Fuci, Sertularia, Corallines, or other moveable bodies, have very seldom prominent papillæ, because they are cleansed by the agitation of the sea like the branched sponges."

Grant not only proved that the currents from the interior of the Spongida were discharged through the oscula and larger orifices; but he also showed that the surrounding water passed into these animals through the innumerable tiny *pores*.

"I first placed," said Grant, "a thin layer from the surface of the *L. papillaris* in a watch-glass with sea-water under the microscope, and on looking at its pores I perceived the floating particles driven with impetuosity through these openings; they floated with a gentle motion to the margin of the pores, rushed through with a greatly increased velocity, often striking on the gelatinous network, and again slackened their course when they had passed through the openings. The motions were exactly such as we should expect

to be produced by cilia disposed round the inside of the pores."

Grant correctly divined the cause of the circulatory currents in the Spongida, but he never saw the cilia, though—rather curiously—he saw and described the cilia of the free ciliated "ova" (as he named these reproductive bodies), which had been swept by the water-currents from the interior of the Spongida out through the oscula. In 1850, Dr. Dobie, at Berwick-on-Tweed, on examining with a $\frac{1}{8}$ th inch objective some gelatinous matter removed from a specimen of *Grantia* (*Sycandra*) *compressa* (Fig. 4, Plate XIV.), which he had previously slit up, "distinctly saw the individual cilia slowly lashing and of extreme tenuity." In Sept., 1850, Bowerbank carefully examined, at Tenby, specimens of the same sponge, about a quarter of an inch in length; he saw particles of excrementitious matter ejected through the large osculum (α , Fig. 4, Plate XIV.) by a forcible and continuous current, and "on examining the exterior of the same specimens the in-current action over the whole surface of the sponge was equally well, though less forcibly demonstrated. The floating particles in the water, when within the action of the in-current orifices, were at first slowly, but afterwards rapidly, drawn towards the sponge, and the action was similar over the whole of its surface, some descending on the upper part of the surface of the sponge, while others ascended to the lower part, with about an equal degree of force. "The fluid," Bowerbank added, "does not appear to enter the sponge by well-defined or regular orifices, but to pass, by numerous irregular pores, through the surface between the outer layer of the spicula." On making very rough transverse sections of this sponge, Bowerbank at last, by careful microscopical examination, saw the cilia lining the radial tubes or chambers (running from the pores through the wall to the central cavity of the sponge) in action. He figured the ciliated cells as shown in the illustration (Fig. 4, Plate XVI.), and described them as "tesselated cells and cilia." Like a good many other observers, Bowerbank failed to see the characteristic part of the cell—the *collar*—as shown in the cells (Fig. 3*a* and Fig. 2*c*, Plate XVI.).

In 1857, Bowerbank showed how the action of the circulatory system of the Spongida could be readily demonstrated. He took a small but vigorous specimen of *Spongilla fluxuatis*, and added

finely-powdered indigo to the water. The particles of indigo could be seen passing in great numbers through the pores, coursing along in different directions through the sponge, and many of the larger particles rushing with excrementitious granules through the oscula. The smaller particles of indigo were found to be mostly retained in the sponge, so that, if the supply of indigo was maintained for an hour, the whole sponge became of a distinctly blue colour, regaining its usual pellucid aspect in about twelve to eighteen hours subsequently, from the digestion in its interior of the particles of indigo, and the ejection through the oscula of all excrementitious matter.

It was also noticed by Bowerbank that the water-currents in the Spongida varied considerably, at different periods, in rapidity, volume, and force. However quiescent a sponge might seem, he usually observed a gentle and continued circulatory action, though the larger *oscula* were lessened in size, and the smaller ones, together with multitudes of the *pores*, quite closed. This gentle circulation is sufficient for the oxygenation of the *cells* and soft gelatinous *sarcode*, which form the living portion of the sponge, sufficient, in a word, for the *respiration* of the animal. For *feeding* purposes, Bowerbank showed that all the *pores* were opened widely, the *oscula* increased in dimensions, the water-currents rapid, forcible, and full, innumerable particles of food being swept through the *pores* into the interior of the sponge, where they adhered to the sticky, sarcodous lining of the many passages, and became digested, the excrementitious and collapsed *débris* being finally ejected through the *oscula*.

STRUCTURE OF SPONGES.

When we come to the structure of Sponges, we enter on debateable ground. In a general way, it may be stated a sponge consists of a mass of transparent or semi-transparent, gelatinous or treacly, sticky and tenacious substance, called *sarcode*, permeated in various directions by the channels of a canal system, which is lined in a greater or less degree by *ciliated* and *collared cells*, and supported by a *skeleton*, consisting either of calcareous, siliceous (or flinty), or keratose (or horny) spicules or fibres. The entrance to, and the exit from, the canal system are both, as has been

already explained, on the surface of the sponge: the former, by innumerable minute *pores*, which the animal has the power of opening and closing; and the latter, by larger apertures or *oscula*, which also can be increased or diminished.

The *ciliated* or *flagellate*, *collared cells*, lining the canal system, form one of the essential elements—Saville Kent insists *the* essential element—of Sponge Structure; and it is this flagellate lining that constitutes the *endoderm* of Haeckel and others, who group the Sponges with the Corals and Zoophytes (*Cœlenterata*). The tenacious, hyaline, and glairy *sarcode*, in which amœbiform cells are distributed, and the skeleton formed, is the *mesoderm* of the Haeckel school, and *cytoblastema* of James Clark, the amœbiform cells (according to the latter authority) being termed the *cell-elements*, and by others *cytoblasts* or *cytodes*. The outer or superficial stratum of the *sarcode*, or *cytoblastema*—in some instances very distinct from the rest of this substance—is the *investing membrane* of James Clark, the *dermal membrane* of Bowerbank, and the *ectoderm* of Haeckel. These different terms imply very divergent views, but it is sufficient to state here that the entire *sarcode*, superficial or deep, of a sponge possesses remarkable vital and contractile powers, pores being opened at various points and closed so as to be unnoticeable, lacerations being readily repaired, and fragments or separations from the main body being capable of change of form and slow movement.

The skeletal structures of Sponges form a basis for their classification; thus there are (1) the *Calcareous Sponges*, the spicules of which consist of Carbonate of Lime; (2) the *Siliceous Sponges*, in which the spicules are of Silica or flinty matter; (3) the *Horny* or *Keratose Sponges*, the skeletons of which consist of a tough horny substance; and (4) the *Gelatinous Sponges*, which possess neither spicules, horny supports, or skeletal structures of any kind.

The "Sponges" used for household purposes are the *horny* skeletons of *Spongia officinalis*.

Halichondria panicea and *H. encrustans* (Figs. 1, 2, 3, Plate XIV.) possess skeletons of *siliceous* spicules, joined together with horny substance.

Sycandra (*Grantia*) *compressa* and *ciliata* (Figs. 4, 5, Plate XIV.) have skeletons made up of calcareous spicules.

We have already alluded to the *Sack Sponge*—*Sycandra*, (*Grantia*) *compressa* (Fig. 4, Plate XIV.). The pores of this sponge open into radial tubes or chambers, which, passing through the thickness of the wall of the sponge, open into the central cavity of the latter. One of these radial tubes, lined with its ciliated or flagellate collared cells, is represented in the accompanying diagram (Fig. 2, Plate XVI.); the arrows indicate the current passing into the tube through the pore (*a*), the water being swept along by the flagellate collared cells (*c*), through the efferent opening (*b*) into the main cavity or cloaca of the sponge, whence it is ejected from the large osculum (Fig. 4, *a*, Plate XIV.). Some spicules (*d*, *e*) are shown in the diagram (Fig. 2, Plate XVI.), but we are concerned chiefly with the flagellate and collared cells. The general plan of this sponge, it will be noticed, is very simple; and the same remark applies to the *Crowned Sponge*—*Sycandra* (*Grantia*) *ciliata* (Fig. 5, Plate XIV.). Our specimen shows a group of four; these are elongated, from one to one and a half inches in length, cylindrical in contour, from about one-quarter of an inch to one-third of an inch in diameter at the thickest part, very slightly stalked, and very gradually increasing in size towards the free end, in which is situated the single osculum. Each therefore is slightly club-shaped, and all are gently curved. The colour is a pearly or greyish white, the entire surface bristly or hispid, and so porous as to be partially transparent when held up to the light. The terminal osculum (*a*) is surrounded by a crown of delicate, brightly-glistening spiculæ about one-tenth of an inch or more in length; and on the specimens being held up to the light and turned slowly round, the general bristly appearance of the sponge is clearly due to similar, though less lengthy spiculæ, which surround the entrances to the innumerable pores. These spiculæ are beautiful examples of *defensive spiculæ*; they encircle every pore, and, during gentle circulatory action, the fringe of each little circle falls in so as to present a pointed cone of bristles against dangerous intruders, whereas, when the currents are vigorous and full, the cones of spiculæ assume, by mechanical means, a cylindrical form. The same description applies to the spiculæ surrounding the osculum (*a*), except that the bristly circle becomes of a more radiate form as the water-current increases in force.

Defensive Spiculæ surrounding the pores are also shown in the diagram of *Sycandra compressa* (Fig. 2, *d*, Plate XVI.). As in the latter sponge, the pores of *S. ciliata* open into radial tubes, which pass through the wall of the sponge to the central cavity.

We have before us a microscopical slide, showing a fine section of the radial tubes; and the accompanying illustration (Fig. 1, Plate XV.) shows exactly and truthfully what is to be seen with a one-twelfth inch objective. The tubes are lined, it will be seen, with *flagellate collared cells*, a few of which have become detached from the others. The cutting of sections for the proper examination of the collared cells is by no means an easy matter, as, however well hardened and prepared, the structural elements of this delicate and fragile sponge are very readily displaced. From the examination of several slides, however, we are convinced that the collared cells are arranged in nature side by side with almost exact mathematical regularity, such as represented in the diagram of *Sycandra compressa* (Fig. 2, *e*, Plate XVI.). We have drawn, on a greatly enlarged scale, two of the detached collared cells (Fig. 2, Plate XV.) just as we saw them, except that the flagella are curved so as to be included in the plate. We saw all the parts represented, viz., the whip-like *flagellum* (*b, b*), the *collar* (*a, a*), the *neucleus* (*c*), and neucleolus, the *granular protoplasm* (*e, e*), and (as far as one could judge from a prepared slide, and the description given by others) also the *contractile vesicles* (*d, d*).

The flagellate collared cells form a very essential part of sponge structure; through their action the circulatory water-currents, necessary for respiration and feeding, are kept up; and they line the canal system, in greater or less degree, and speaking generally, according to one or other of two broad plans, throughout the whole of the Spongida. In some Sponges, the flagellate collared cells line more or less completely the entire canal system; in a great number of others, they line only rounded cavities within the sponge, to which the water runs from the pores through "afferent" channels, and from which the currents are conveyed by "efferent" canals to the oscula. *Sycandra compressa* and *Sycandra ciliata* (Figs. 4 and 5, Plate XIV.) illustrate the first plan, the entire length of the radial tubes being lined by the collared cells (Fig. 1, Plate XV., and Fig. 2, Plate XVI.). As

regards the second plan, Lieberkühn showed in 1856 that the flagellate cells in *Spongilla* were limited to certain dilations, or *ciliated chambers*, of the canal system. In 1857 Carter also found that, in an Indian species of *Spongilla*, the flagellate cells were only found as a single lining of small rounded cavities, which he called *ampullaceous sacs*, excavated in the sarcode of the Sponge. James Clark, in 1871, described similar cavities lined with flagellate cells, under the title of *monad chambers*, in the American *Spongilla*. "A similar ampullaceous disposition of the collar-bearing cells," observes Saville Kent, "is found to obtain among a very extensive series, if not throughout the majority of the Spongida; in fact, all the known members of the *Myxospongia* (gelatinous sponges), the greater part of the *Silicospongia* (siliceous sponges), and in accordance with the representations given by Professor Haeckel, the family of the *Leuconidae* among the *Calcispongia* (calcareous sponges)." The accompanying diagram (Fig. 1, Plate XVI.) represents a vertical section of *Halisarca*, the jelly sponge; and here we see spheroidal *ciliated* or *monad chambers*, or *ampullaceous sacs* (*b, b*), the *afferent canals* leading into them from the pores (*a, a*), and the *efferent canals* (*c*) passing from them to an osculum. Saville Kent, in Vol. III. of his "Manual of Infusoria," gives an illustration of "*Esperia*, a siliceous-spiculed sponge, in vertical section, showing grape-like arrangement of the ampullaceous sacs round a single afferent or pore system."

There can be no doubt, from the facts related, of the essential importance of the flagellate collared cells in Sponge structure, whether considered as the *endoderm*, or lining, of digestive cavities or simply as Infusoria associated together in compound colonies. Those who advocate the latter view, and especially Saville Kent, lay great stress on the exact resemblance or identity of the collared cells of the Spongida with the independent collared animalcules (*Choano-flagellata*). Here is a diagram (Fig. 5, Plate XVI.) representing an independent collared monad—*Mono-siga gracilis*—as described by Saville Kent. This animalcule is found attached by a slender stalk to zoophytes and sea-weeds; and it is possessed of a flagellum (*b*), collar (*a*), nucleus (*d*) with nucleolus, and contractile vesicles (*e, e*), all of which are found,

for example, in collared monads from *Sycandra compressa* (Fig. 3 a, Plate XVI.), and *Sycandra ciliata* (Fig. 2, Plate XV.). Saville Kent describes the *collar* (Fig. 5 a, Plate XVI.) as an organ of great importance, which can only be satisfactorily studied by means of the highest objectives and strongest illuminating apparatus, at the same time that the animalcule is supplied with artificial coloured food, such as powdered indigo or carmine. Under such conditions, "it will be found that the collar consists of a transparent infundibuliform film of sarcode, that may be protruded from and withdrawn at will into the general substance of the monad's body, in the same manner as the sarcode prolongations or pseudopodia of an *Amœba* or other Rhizopod. As in the pseudopodia of certain Rhizopods, such as the Foraminifera, it will moreover be found that, notwithstanding the extreme tenuity of the sarcode films, a circulation of its substance is being constantly maintained; flowing upward on the outside, over the distant edge, and downwards on the inner surface, at the base of which it again comes in contact and merges with the protoplasmic substance of the body. The wine-glass-like film of sarcode doubtless acts as an efficient branchial or respiratory organ, but such by no means represents its most important function. In conjunction with the centrally-enclosed flagellum it constitutes a most admirably contrived trap, or snare, for the capture and retention of the animalcule's food. Whirling round with inconceivable rapidity, the last-named organ, the flagellum, creates a strong centrifugal current in the water, setting in from behind towards the direction of its own apex, and bringing with it all such tiny organic particles as do not possess sufficient weight or power to stem its tide. But for the outstretched collar, these would simply hurry with the stream past the monad's body, and out of reach. Not for them, however, so easy a passing of the rapids! In the midst of their swift career they strike against the almost impassable films of sarcode of which the organ is composed, and to this they adhere as tenaciously as a snared bird to a lime-daubed twig, or an incautious fly to a spider's web. Then slowly, almost imperceptibly, the captive atoms are carried along with the circulating current of the collar's substance up the outside, and down the inside, until, on reaching the base of its inner

surface, they are engulfed within the sarcodous substance of the monad's body (see *f* and arrows, Fig. 5, Plate XVI.). The food-particles after ingestion are gradually accumulated into spherical agglomerations (*c*, *c*), and then regurgitated through the body under conditions nearly identical with those exhibited by such a higher improvised type as *Vorticella*. The indigestible residua are eventually liberated from the area limited by the base of the collar, within which they previously gained access."

Other important characteristics are the presence of two or more contractile vesicles (*e*, *e*) situated in the under portion of the monad's body, and the presence of a well marked nucleus (*d*) or nucleolus. Now, Saville Kent insists that the flagellate collared cells in the Spongida (see Fig. 3 *a*, Plate XVI., and Fig. 2, Plate XV.) correspond, "structurally and functionally, in every detail," with the independent collared Infusoria. "The collar," he says, "in either case presents the same structure and functions, exhibits the same circulatory currents or cyclosis, and acts in a precisely similar manner as a trap for the capture of food. The body contains an identical, centrally-located, spheroidal nucleus or endoplast, and a corresponding, posteriorly located series of rhythmically pulsating contractile vesicles." There are other remarkable points of resemblance. The independent monads are capable of extraordinary changes of form, retracting both collar and flagellum, throwing out sarcodous processes or pseudopodia, and assuming various amœbiform aspects. So it is with collar cells of the Spongida. Saville Kent points out that the dissections of a living Sponge, and examination of the flagellate cells, should be proceeded with quickly, as the cells are apt to soon withdraw collars or flagella, or both, throw out variously shaped sarcodous processes, and take on the most curious shapes (Fig. 3, *a*, *b*, *c*, *d*, *e*, *f*, *g*, *h*, *i*, Plate XVI.). These amœbiform cells may also re-attach themselves to a spiculum of the Sponge, withdraw their pseudopodic extensions, throw out once more collars and flagella, and throw out also a thin bed or investment of sarcodous material.

Certain genera of the Flagellate Infusoria, as *Phalansterium* and especially *Protospongia*, are very near to the Spongida as regards their condition of colonisation, as the monads are asso-

ciated together and embedded in a gelatinous material excreted by themselves, the flagella and collars being the only parts projected into the surrounding water. In the gelatinous bed of a colony of *Protospongia* may be seen at the same time fully-developed collared and flagellate monads, and monads with collars and flagella withdrawn, some irregularly shaped and amœbiform, some dividing, and yet others breaking up into spores.

The glairy, tenacious sarcode of the Spongida corresponds to the gelatinous bed of the colonised Infusoria, such as *Protospongia* and allied genera. Distributed within this sponge sarcode, or *cytoblastema*, are numerous amœbiform *cell-elements* or *cytoblasts*, which repeatedly change their form, move about from one part to another of the sarcode, and by their action in the superficial or peripheral layer of the latter (in which they are particularly abundant) bring about the closing and opening of the pores and the enlargement and diminution of the oscula.

As in the colonised flagellate collared Infusoria, so in the Spongida are the collared cells found to retract flagellum and collar, some becoming amœbiform, sending out variously-shaped pseudopodic extensions and moving about in the cytoblastema; others, again, either singly or after coalescence with other cells to form larger bodies, assuming a quiescent state, then breaking up by segmentation into rounded spores, which go to increase the general sponge mass. "It is only requisite to point still more emphatically," says Saville Kent, "to the fact that these spores, distributed broadcast throughout the substance of the cytoblastema, may, as ascertained by the author, be met with and traced onwards through every intermediate size and stage, from the single spheroidal spore up to the adult collared monads or amœbiform cytoblasts; the derivation of these spores through the splitting-up into a granular or sporular mass of the entire substance of the matured collar-bearing zooids being correspondingly substantiated." The reproduction of the Spongida is effected, according to Saville Kent's investigations, in a somewhat similar fashion. The collared cells, with collars and flagella retracted, become amœbiform, pass into the cytoblastema, coalesce into larger ovoid bodies, divide by segmentation into a swarm of ciliated or flagellate monads, in which state this so-called "ciliated larva," or "swarm-gemmule," passes

through the oscula of the sponge into the surrounding water. "In their most characteristic form," writes Saville Kent, "these reproductive bodies, or cell-aggregates, consist of a uniform series of collared zooids; but by irregular growth one-half may arrive at or pass maturity in advance of the other, the product then being a compound structure, presenting a close correspondence with that phase of development of the Metazootic ovum known as the amphiblastula. Since, however, these bodies are in no way comparable with the Metazootic ovum—not being the product of the concourse of true sexual elements—the above likeness is simply homoplastic, and the body as a whole, consisting as it does of an aggregation of numerous independent zooids, may be most appropriately denominated a 'swarm-gemmule.'"

For want of space, we cannot dwell further on this interesting subject; but the few remarks offered by us suffice to show the sharp divergence of opinion that exists among biologists as to the real position of the Sponges. The views advanced by Haeckel in the first instance, that the sponge-body can be shown to consist of an ectoderm, mesoderm, and endoderm, that spermatozooids are present in the Spongida, and that the reproduction of this interesting group of animals takes place by means of the development and segmentation of true ova or eggs, are all stoutly and diametrically opposed by Saville Kent and those who think with him. The latter authority argues his case, we think, with great force and consummate ability in the article "On the Nature and Affinities of the Sponges," and in other parts of his "Manual of the Infusoria"; and supports his views with beautiful drawings illustrative of his own personally conducted investigations.

The questions at issue require for their solution a careful, minute, and laborious investigation of the vital microscopic elements of sponge structure, and especially of the origin, life-history, and development of the reproductive bodies of this group of animals. To those who have leisure, and a leaning towards biological pursuits, here is a fine field open for the display of industry and skill in delicate scientific research.

EXPLANATION OF PLATES XIV., XV., XVI.

PLATE XIV.

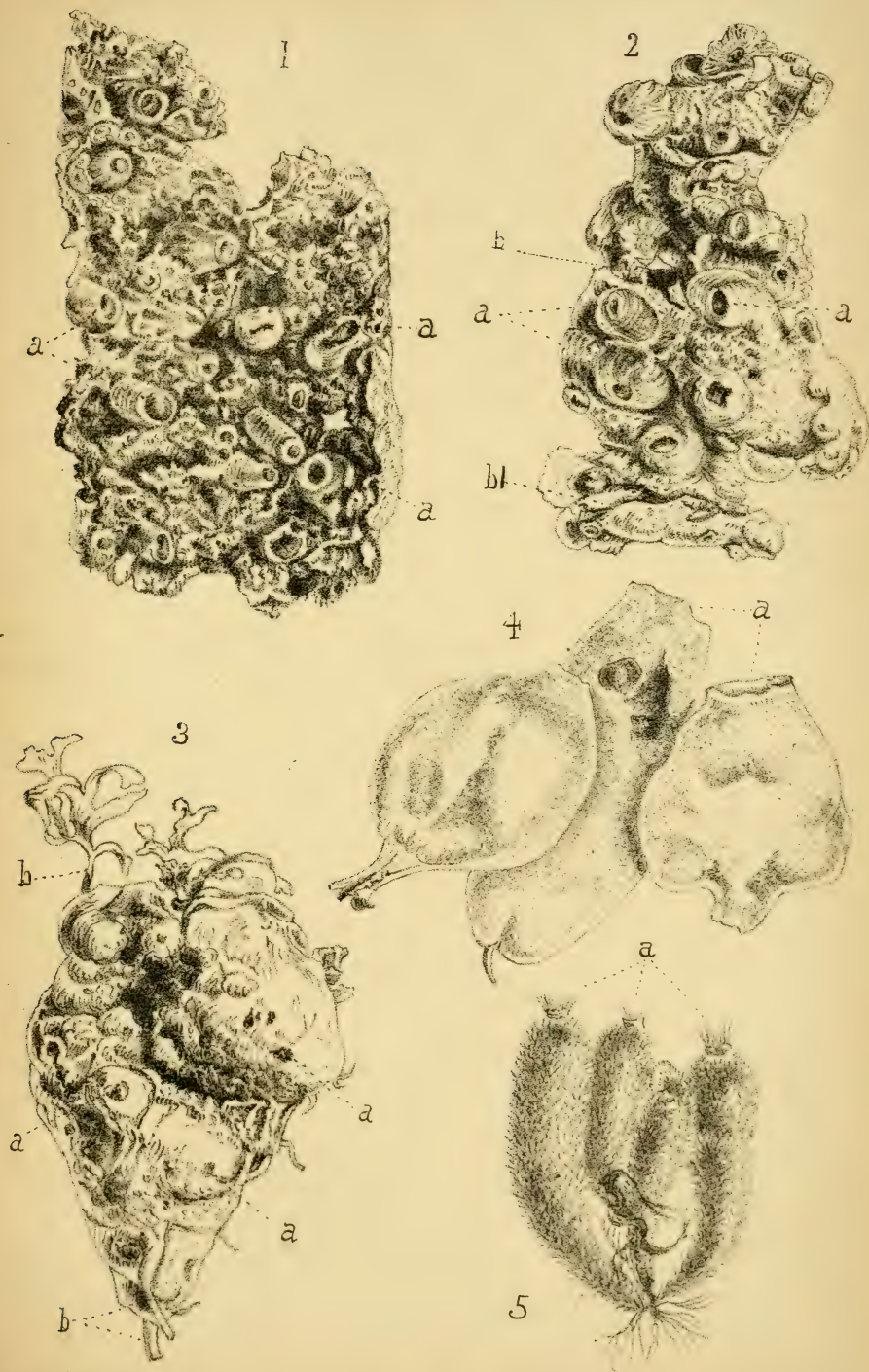
- Fig. 1.—*Halichondria panicea*, the Crumb-of-Bread Sponge, taken off a piece of rock ; *a*, *a*, oscula, or fecal orifices.
- „ 2.—*Halichondria panicea*, the Crumb-of-Bread Sponge, growing on and around sea-weed (*Fucus*) *b* ; *b* 1, channel in sponge once filled by seaweed ; *a*, *a*, oscula.
- „ 3.—*Halichondria encrustans* growing on and around sea-weed (*Chondrus*), *b*, *b* ; *a*, *a*, oscula.
- „ 4.—*Sycandra compressa*, the Sack Sponge ; *a*, oscula.
- „ 5.—*Sycandra ciliata*, the Crowned Sponge ; *a*, oscula.

PLATE XV.

- Fig. 1.—*Sycandra Ciliata*, the Crowned Sponge. Part of longitudinal section, showing collared flagellate cells lining radial tubes, and a few spicules, as seen with a 1/12th inch objective.
- „ 2.—Isolated collared flagellate cells, greatly magnified, and drawn from the same slide as above. *a*, *a*, collar ; *b*, *b*, flagellum ; *c*, nucleus, containing nucleolus ; *d*, *d*, contractile vesicles ; *e*, *e*, granular protoplasm.

PLATE XVI.

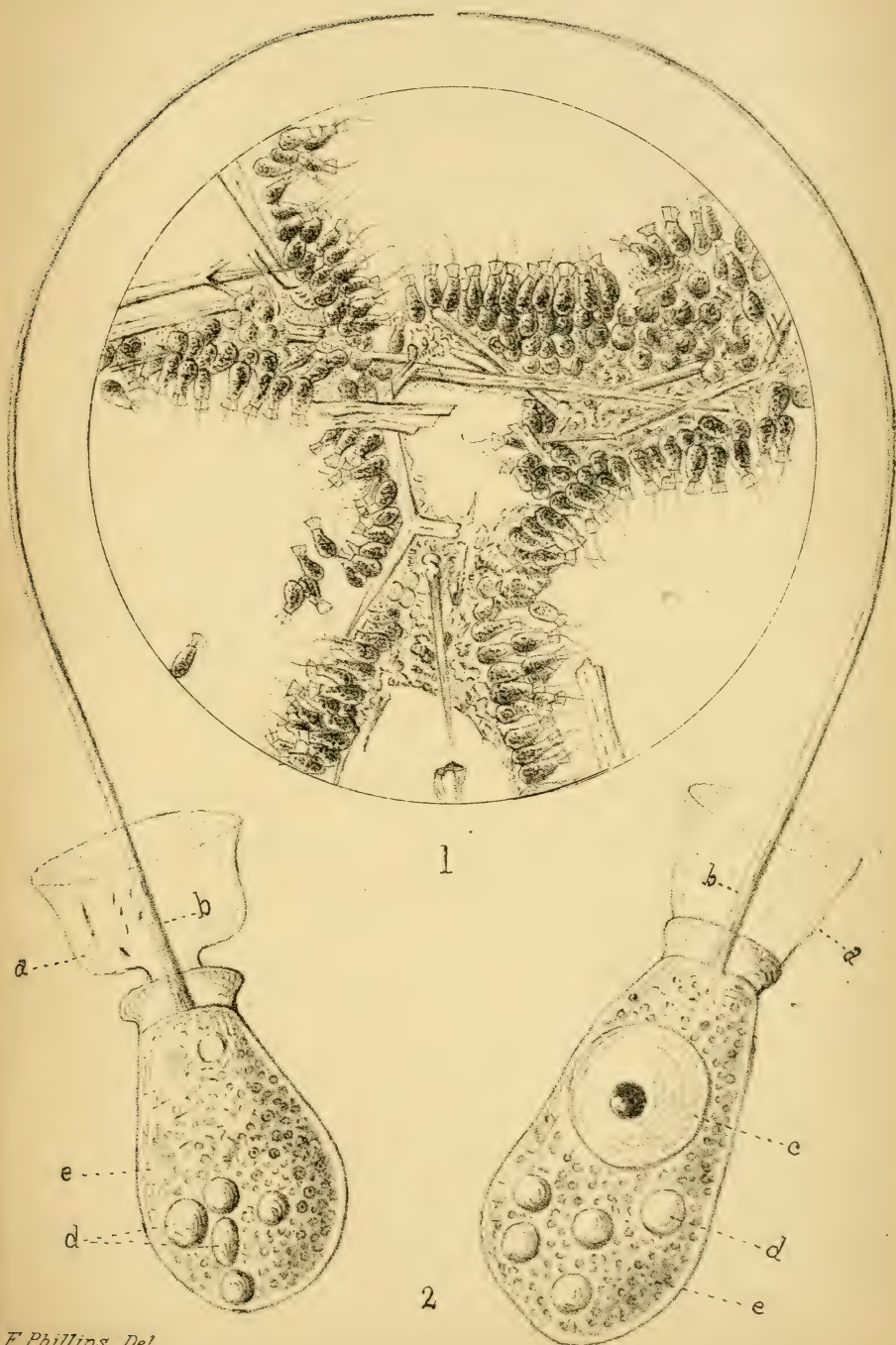
- Fig. 1.—*Halisarca lobularis*, diagram of vertical section. *a*, *a*, Pores or apertures of afferent canals ; *b*, *b*, ampullaceous sacs, monad chambers, or ciliated chambers ; *c*, efferent canal leading to osculum.
- „ 2.—*Sycandra compressa*, diagram of transverse section of Sponge, showing one entire radial tube or ciliated chamber. *a*, Pore through which water enters ; *b*, efferent channel opening into central cloacal chamber ; *c*, flagellate collared cells lining radial tube or chamber ; *d*, external defensive spicula ; *e*, internal triradiate spicula.
- „ 3.—Diagrams of flagellate collared cells from *Sycandra compressa*, in various stages of metamorphosis. *a*, cell, with collar, flagellum, nucleus (*j*) and nucleolus, and contractile vesicles (*k*) ; *b*, *c*, cells in different stages of metamorphosis ; *d*, *e*, groups of cells in different stages of change, collars and flagella being retracted in some, extensions of sarcode into processes, or pseudopodia, occurring in others, and granular food being ingested (*l*) in some ; *f*, *g*, *h*, *i*, cells variously metamorphosed and amœbiform.
- „ 4.—Detached ciliated cell from *Grantia* (*Sycandra*) *compressa*, as described by Bowerbank.
- „ 5.—Diagram of a flagellate collared independent monad, *Monosiga gracilis*. *a*, collar ; *b*, flagellum ; *c*, *c*, ingested food granules ; *d*, nucleus, with nucleolus ; *e*, *e*, contractile vesicles ; *f*, particles of food.



F. Phillips. Del.

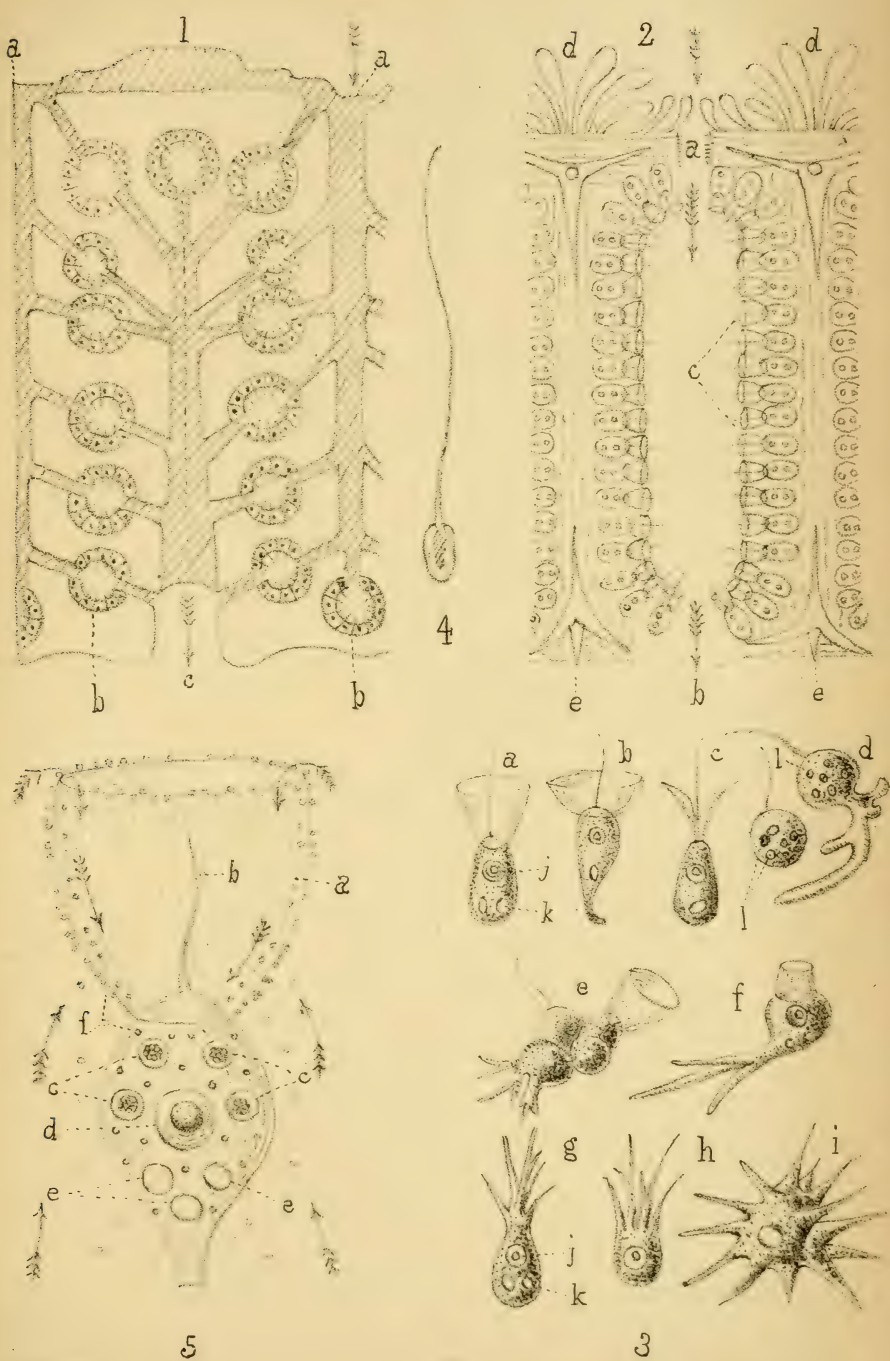
Halichondria panicea *H. encrustans*





Sycandra ciliata





F. Phillips.

On the Cultivation of Diatoms by Artificial Means.

BY DR. P. MIQUEL. (Translated from *La Diatomiste*.)

CHAPTER III.

CULTURE OF DIATOMS UNDER THE MICROSCOPE.

WHAT I have already said respecting the ordinary cultivation of Diatoms will admit of my being very brief respecting the propagation of these algæ under the microscope, for the culture of Diatoms in cells is conducted in a manner identical with that which the operator has practised in the laboratory, whether in a glass vessel or in other receptacles. But I ought to add that if the manipulations required by the cultures under the microscope are a little more difficult, they require special arrangements and cells of a peculiar form, permitting—

First—The keeping easily during many months the fecundated macerations.

Second—The observation of the living Diatoms with objectives of the lowest powers as well as with the most powerful immersion objectives.

Third—The photographing the general appearance of the growths, and the phenomena of the life of the Diatom.

Optical arrangement.—This arrangement has been made, according to my instruction, by Alf. Nachet. It is composed of a horizontal, photo-micrographical arrangement of short length, provided for the observation and study of the cultures, with a tube carrying the objective perpendicular to the optical axis, in which a prism of total reflection directs the luminous rays at will. This microscope, adapted both for study and photo-micrography, admits of all kinds of illuminations, and especially of a large achromatic condenser with a focus of from three to four centimetres, capable of transmitting very intense luminous rays through the cells ; in a word, strongly lighting up the objects that are to be reproduced by photography. The cells containing the culture are held on a plate, movable and vertical, while the interior is examined through a cover-glass of about 0·15 mm. of thickness.

I have so fully described the microscope in the *Annual of the*

Montsouris Observatory for the year 1892—93 (page 544, etc.), that I need not describe it further here.

Culture Cells.—For the culture of Diatoms I make use of two kinds of cells.

The first kind consists of an object-glass, on which is cemented a ring of glass, ground on both its faces, of 5 mm. in height and 24 mm. of exterior diameter (Fig. 1). This ring is pierced at its upper part with an opening, permitting the introduction of liquids and sowing them. On the upper face of the ring is cemented a thin cover-glass of over two-tenths of a centimetre thick. You thus have a little vessel of two centimetres capacity, where the cultures can be carried on as well as in those of many times its volume. The situation of the aperture on the top of the ring does not allow this little aquarium to be kept in any but an upright position. Thus, for the culture of certain Diatoms, especially those that seek the light, you expose the cell with the cover-glass towards the light. At the end of a certain time, varying from weeks to months, the interior of the glass is covered with a light yellow film of living frustules. With the *Nitzschia longissima*, for example, which the microscope reveals, the result is truly enchanting.



Fig. 81.—Microscope Aquarium, half the real size.

L L, Glass Slip, 3×1 ; O, Aperture in ring; E E, Culture.

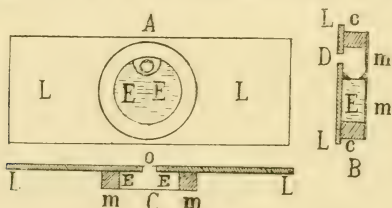
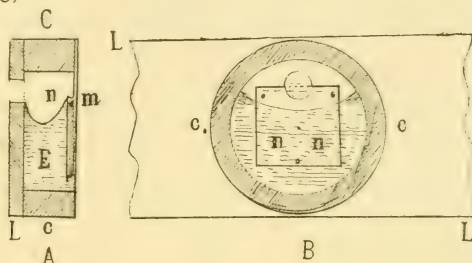


Fig. 82.—A, Cell as seen on the microscope, half size. L L, Glass Slip; E E, Cell.

B, Cell, transverse, half size. L L, Glass Slip; D, Opening of 2 mm.; c c, Ring; m m, Cover-glass; E, Culture.

C, Cell, longitudinal, half size. L L, Glass Slip; O, Aperture; m m, Cover-glass; E E, Interior of Cell.

As many other Diatoms develop by preference in the bottom of the vessels, this peculiarity requires special arrangements for observing them. To meet this, I have constructed a new cell (see Fig. 83).



.Fig. 83.—*A*, Culture Cell with capillary plate, transverse, full size.

L, Glass Slip; *c c*, Ring; *m*, Cover-glass; *n*, Thin glass interior; *E*, Culture.

B, Cell seen on the microscope, full size. *L L*, Glass Slip; *c c*, Ring;

n n, Interior thin glass, supported on three spherules of mastic.

On a glass slip, which is bored towards its upper edge with a hole about 2 mm. in diameter, there is cemented, by means of mastic, an unbroken ring, and on the ring a cover-glass. In this way you have a new cell, of which the aperture is lateral, which can be held vertical during observation, but can be laid in a horizontal position during the culture, with the cover-glass downwards.

Thus the deposits, instead of forming in the lowest parts of the ring, remain and encrust the cover-glass, and the Diatoms that are sown in the little cells are born and multiply on the inner face of the cover-glass, which admits of following their growth for many months, with objectives of all sorts.

This arrangement also admits of your treating the algæ with different re-agents that can be introduced by the lateral opening without deranging the cell or its contents, and by means of special instruments it is easy, when the microscope I have devised is used, and by means of a condenser of long focus, to act on the Diatoms with needles or bristles, so as to render the study of them more easy.

In order to observe the multiplication of some species and protect them against the lowering of the liquid, which may result from the evaporation of the water in the cell, and to keep them within the range of high-power objectives, I have adopted the

plan of a second thin glass in the inside of the ring, fixed to the cover-glass at some hundredths of a millimetre distance (see Fig. 3). By this means the level of the liquid may be considerably lowered and agitated with more or less force, without the risk of drying or displacing the creatures that are growing between the plates.

Plan of Cultures in these Microscope Cells.—The cells, filled with water and charged with a very small portion of bran, are exposed for an hour in a water-bath to a temperature of 70° C., to destroy all pre-existing germs of algæ or infusoria. This done, and the cell cooled down, you sow, by means of a pipette with a point, drawn out and bent, a little of the liquid of a culture already formed, and leave the cell lying on the glass cover, with the glass slip uppermost. The whole is placed on a plane slightly inclined, and covered, according to its needs, with paper—white, black, or coloured. The inclination of the plane or of the suspending apparatus ought to be such that the bubble of air in the cell should open to the circular aperture formed in the glass slip.

At the end of some days the interior surface of the cover-glass will be covered by a light deposit, where the Diatoms stir and freely multiply in filaments if they are moveable ; or they multiply in various other manners if they are immovable (*Melosira*, *Bidulphia*, *Fragillaria*, *Meridion*, etc.).

The liquid that evaporates from the cell is replaced with boiled distilled water by means of a capillary pipette. This need only be done every eight days in the heat of summer and not more than once a fortnight in winter. It is well to place above the opening in the glass slip a little circular bit of flannel, as much to lessen the evaporation as to preserve the maceration from contact with atmospheric impurities.

You may thus cultivate, during six, eight, or even ten months, the same species ; follow all the different phases, from the first appearance of the Diatoms after they have been sown, to the formation of the auxospores, and ultimately to the decay of the frustules ; you may also fix, by means of photography, all the interesting phenomena that present themselves. I need not repeat that the arrangements of my cells admit of treating the contents with poisonous substances and observe their reaction, and then

what are the phenomena they present at the moment when the Diatoms succumb to their action.

It is often preferable to fill the cells with the liquid and a trace of deposit from a culture. By this means you obtain very quickly a very flourishing growth in the cell, identical with that which has been going on in the mother liquid.

The movement of Diatoms is, in some cases, very difficult to observe for a length of time. This is an inconvenience to which it is, and probably always will be, difficult to apply a remedy ; but the diatomist ought to esteem it a fortunate thing that the algæ that are the objects of especial study have movement infinitely less lively than the Protozoa, the study of which, thanks to the labours of Balbiani and of Bütschli, is nevertheless at the present time in an advanced stage.

CHAPTER IV.

THE FUTURE OF THE CULTURE OF DIATOMS.

FROM the commencement of this series of articles, I had the intention to recapitulate the principal facts that the culture of Diatoms has revealed to the observer ; but in proportion as I have advanced in these studies, the field, at first relatively narrow, has widened so rapidly that I have been obliged to preserve in another publication,* in numerous paragraphs, the interesting remarks that the cultures in question have enabled me to note. I have therefore thought that it will be more profitable for the readers of this Journal to find in the special monographs the facts that contribute to the history of such and such Diatoms, and to read the general conclusions, in which all the interest resides, especially in the exposition of what has gone before.

I prefer to give, in broad features, in the lines that follow, some special methods of investigation, pointing out the ends that may be attained. In thus acting I believe I shall do more useful work, more profitable to the science of the siliceous Phéophyces than in the enumeration of the general properties of these algæ ; a kind of abridged edition of the labours that I have already published and of those to which I have consecrated these numerous pages.

* *Annales de Micrographie*, Tome IV., 1892.

Normal Cultures of Diatoms.—We have seen in the foregoing three chapters the methods of carrying on these cultures, which are those where the evolution of the Diatoms takes place in a regular and natural manner, with the appearances that they present in the media where they are commonly found.

The utility of such cultures does not need to be shown; besides, the possibility of keeping at home at all seasons of the year living Diatoms of the most varied kinds, the herbaria of diatomists are by it remarkably simplified. Indeed, in his excursions, the observer only needs to take sowings—the taking with him extensive receptacles is often very useless, especially those ingenious sacks or those grotesque knapsacks; thirty little tubes, such as are used for pilules—previously washed in boiling water, contained in a box or portfolio, are, with pincers, all the necessary tools.

Lastly, my comrade, the Surgeon-Major Couteand, sent in the expedition of the *Manche*, conducted by the commandant Benamié—an expedition which visited during last summer the Shetland and Feroe Islands, Iceland, the island of Jan Mayen, and Spitzbergen—has brought me 112 small tubes of various deposits, both of fresh and sea water, taken from different places. Many of these deposits could not be put in culture for fifty or sixty days after they were collected. The result has surpassed my expectations, and I have obtained by the methods that I have described upwards of two hundred species of Diatoms, of which I have made numerous preparations, to serve as a basis for a work that ultimately I shall publish, with the help of my learned friend, Dr. Couteand. Here is one of the first results of these processes for the culture of Diatoms:—

Abnormal Culture of Diatoms.—I have given this name to cultures where the nutrition is slightly or largely modified, whether by means of chemical elements or by physical agents, favouring, as the case may be, either the development or the suppression of the diatomic life.

The anomaly may be dependent on physical agents. Indeed, Diatoms raised under artificial light may, according to their nature, give very fine or very poor results. Many of these algæ accommodate themselves very well to a feeble light (a jet of gas burning from fifty to sixty litres per hour); of this number many Nitzschias,

Melosiras, and Cyclotellas ; whilst there are others that accept this feeble light as a *pis aller* ; others vegetate little with all the light from gas. These conditions of invariable light do not occur in nature. Thus, I reckon the culture of Diatoms and green Algae by gaslight or under the action of other sources of artificial light as abnormal.

If, in carrying on the culture of Diatoms under the variable action of daylight you modify the temperature ; if, for example, you raise the temperature to between 30° and 35° C.—a temperature that running waters in our climate never acquire—you will observe with some species an exceedingly rapid development. The *Nitzschia palea*, left at 32° and 33° C. under the action of a bright light, increases so quickly that twelve hours after sowing in a maceration nutrified with straw, its development is easily visible to the naked eye, while many of the cryptogams and the bacteria will not show at all in so short a time in the maceration in which they are sown. The *Nitzschia palea*, in thus developing, sets up a very abundant disengagement of oxygen. The bubbles of gas rise from the bottom of the vessel as thickly as the bubbles of carbonic acid from an alcoholic fermentation in good action, and, by adapting a conducting tube to the neck of the flask that contains the maceration, covered with froth, you may collect in five or six days more than a litre of oxygen almost pure. Many Diatoms placed under like conditions would never develop. It is found that the temperature most favourable to their prompt increase is very low—about from 5° C. to 6° C., a temperature equally abnormal for the Parisian climate.

In modifying the intensity and nature of the luminous radiations—in other words, making them strong or feeble, red, yellow, or blue—you make a change in the speed of the growth of Diatoms, you retard or accelerate, and other things being equal, obtain growths differing greatly from those obtained under white light. These results ought also, in my opinion, to be considered as curious, and perhaps useful, anomalies.

But where the field of research becomes immensely vast—indeed, indefinite—is when, leaving the domain affected by physical agents, you turn to the innumerable chemicals to impart to the cultures special features altogether distinct from those that you note in the normal media.

Add to the macerations hydro-carbons, sugar, glycerine, alcohol, salts of organic acids, and the endochrome of yellow Diatoms becomes habitually colourless ; under the influence of some substances it becomes and remains green. It seems in the first case that the soluble hydro-carbonaceous aliments admit of the Diatoms living without chlorophyll ; that is to say, to live in the absence of the obscure substance, which enables vegetables directly to assimilate the carbon from carbonic acid.

Thus it is, mainly by the help of saline substances, that you obtain results most worthy of note. It is with the salts of minerals—(chloride of sodium, of potassium, of magnesium, sulphates, alkaline phosphates, alkaline earth, alkaline bicarbonates, etc.)—that you most quickly obtain cultures that I have termed “tetralogical,” where the Diatoms lose the usual form, to take others extremely bizarre—without doubt the action of intoxication, or, rather, the loss of the vegetative sense, called by some authors “*Folie du noyau*.”

Is it not surprising that a *Synedra* or a *Nitzschia*, which has been placed in bi-carbonate of soda or in an excess of sea-salts, provided with valves whose faces are undulated, divide themselves up into oblique sections, thus giving two, three, and four strange forms ; whilst in ordinary macerations, with very rare exceptions, these species re-duplicate themselves with the greatest regularity ?

Lastly, M. Tempère places before the readers of *La Diatomiste* the problem, “Are Diatoms of marine or fresh-water origin ?” I believe that it would be unsafe to give a direct reply to this question, based on the inspection of the deposits of fossil Diatoms and of the species now living on the surface of the globe. I think that the solution of this problem, which has a retrospective interest, will be most effectually reached in the laboratory, for it is easy to measure the progressive effects of saline solutions on the Diatoms, and to follow the acclimatisation of these algæ in media more or less charged with salts, or subjected to increasing temperatures. Nothing, then, should hinder us from hoping that it will be possible to arrive at last at the production of forms that have never yet existed, and again to see fossil species, by realising the conditions which prevailed during the multiplication of species, actually lost. The re-habilitation of fossil Diatoms by retrograde

rations, from those living ones that we do possess, if ever it be possible, will enable us to reply in certain manner to the question (possibly premature) of the editor of *La Diatomiste*.

Cultures in Series.—Along with cultures with media differently composed (termed abnormal) cultures may be attempted having special objects in view. These have for their object the multiplication of some forms of Diatoms by the addition to a pure state of such and such algæ; by this means I obtain easily Schizonemes in long branching thalli. These mixed cultures are very interesting to carry on, but their description in the general work would carry us too far. I prefer to speak on the mode of culture in series.

A diatom cultivated in a normal medium increases according to a certain law, easily verified by the living dissociate species. The individual sown being of a known shape, you prove that the number of the frustules of the same shape which proceed from it are as the terms of the expression $(1 + 1)^n$ carried out.*

When in this case the Diatoms of medium size are much the more abundant, in transferring a small number of frustules into new macerations, you ultimately eliminate the large-sized Diatoms, and obtain at the end of a time more or less long, after the tenth, twentieth, or thirtieth successive cultures, Diatoms of very reduced size which are in this alternative, either many kinds disappear if they continue the multiplication, or become the starting point for the re-establishment of the size of the species. Diatoms arrive at their minimum of smallness by way of cultures in series, growing in a very great proportion, and furnishing numerous auxospores, which you meet with by millions in a culture of a dozen cubic centimetres of volume.

In employing this method of culture, I have helped to re-establish the size among many *Melosiras*, the *Nummuloides* and the *varians*, the *Cyclotella comta*, two *Biddulphias* (the *rhombus* and the *aurita*), and that whether by natural or artificial light. With the *Melosira varians* I have lately succeeded, in mid-winter and with a very low temperature; the size in the proportion of one to three, the intermediate and the diametrical division of the large spheres, which are produced in the articulated chains of the

* See *Annales de Micrographie*, Vol. IV., p. 532.

Phéophycée. These re-establishments of form, which I have not noticed to be accompanied with phenomena of conjugation, are produced in the laboratory in seasons the most diverse, which shows that, perhaps, the influence of seasons on the production of sporangious frustules is exaggerated.

I shall have to wait a long time before I can make public the different investigations that I have thought of; in the meantime, I have thought that these methods and these results, all imperfect as they are, ought to be brought to the knowledge of diatomists, with the sole object of advancing the science of Diatoms in a novel way: that of direct experiment. During the carrying out of the work, I do not allow myself to be disturbed, either by the preliminary communication of some, nor by the conclusions of those persons who look upon the Diatoms as their own property, and in which department they have not for long years said anything that was not contrary to truth, or nothing to instruct that was not open to question. Modest amateur, I repeat it. I confine myself, in conclusion, to thanking those who have had the patience to read my essay, and to hope that the hours of leisure that I have devoted to the study of Diatoms have not been entirely lost.

GROWTH OF WILLOW TREES.—*Garden and Forest* has received a photograph of a willow tree standing in Waterbury Centre, Vt., the trunk of which measures twenty-four and a-half feet in circumference, and whose symmetrical top shades an eighth of an acre of ground. A person who knows the early history of this tree testifies that in 1840 it was a tree about six inches in diameter, which had grown from a walking-stick driven into the ground a few years before by some children. In that year it was cut down deep into the ground in the hope of killing it, but it started a new growth, and has reached its present diameter in fifty years. The rapid growth of the willow in favourable localities is well known, and Dr. Hoskins (from whom the photograph was received) writes of another near his home, which sprang from a cane carried by a returning soldier in 1866, and thrust into the soil in his door-yard. It is now more than four feet in diameter, with an immense top, and bids fair, at an equal age, to reach the dimensions of the one spoken of.—*Popular Science Monthly*.

Some Hardening Agents : Their Special Properties and Methods of Using.*

BY PROF. V. A. LATHAM, D.D.S., F.R.M.S.,

*In charge of the Microscopical Laboratories of the North-Western
University; Women's Medical School; Curator of the Museum;
Professor of Histology, Pathology, and Bacteriology,
American College of Dental Surgery;
Late Assistant in Pathology, etc., University of Michigan.*

THE most essential point in Microscopic Investigation is the proper hardening of the material to be examined ; and this must be done gradually, for if any tissue is placed in a strong solution, the elements of which it is composed shrink at once and it is impossible to form a correct idea of their nature.

It is quite as important that pathological specimens should be as thoroughly hardened as normal tissues, but how seldom is this done !

In the first place, it is difficult to get the morbid tissues fresh enough, and yet they are often put on one side, or at most placed in the lump in a small quantity of methylated spirit and water, and it is expected that good sections can then be prepared from them. Nothing is more erroneous than this idea. The subject has probably been dead twenty-four hours at the least when the autopsy is made, or perhaps longer, and, in summer especially, this means utter ruin to such organs as the spleen. How important is it, therefore, that such organs should be put in the hardening medium whilst as fresh as possible.

For this purpose jars of proper size, and with the necessary fluid, should be taken to every post-mortem examination, and bits of any organ that may on any account seem interesting should be put into it. Tie a small label on the various pieces, and they can be separated afterwards. For labels I prefer to use small pieces of tin with numbers punched on them, and a hole in, to tie to each specimen, and number in a book to correspond with them. The specimens may with advantage be allowed to remain in Müller's fluid for a week ; they are then cut into small pieces and

* Read before the Illinois State Microscopical Society.

placed in separate bottles, duly labelled. The same remarks apply to tissues removed by operation. They should be placed in the hardening fluid at once *without more handling than is absolutely necessary*.

If the tissues are placed in a strong solution—such as a one per cent. solution of chromic acid or in alcohol—the elements of which they are composed shrink on the outside and undergo such alterations as render it impossible to form a correct idea of the pathological change that has taken place. Bad hardening and staining, combined with imperfect histological knowledge, will account for most of the extraordinary fallacies that have been made public of late years.

Although in this country (U.S.A.) autopsies are obtained with difficulty, still many are made, and the results are rendered useless by the bad hardening of the material and other causes. The amount of pathological material annually wasted in this way is enormous, and the hindrance to the advancement of our knowledge of disease is very great.

The object of hardening is not only to give the specimens greater consistency, so that thin sections may be more easily obtained and more safely manipulated, but also to fix the tissue-elements as far as possible in the same relative position as in the living body. The hardening process also acts on the protoplasm of the cells and prevents their swelling up when placed in water and various staining fluids. The hardening fluid used must be one which will not itself injure the specimen, and which can be thoroughly removed by washing in water, in order that it may not interfere with the staining operation.

Hardening may be accomplished either by freezing or by various chemical re-agents. The latter act by coagulating the albumen, by withdrawing the water, or, in some instances perhaps, by combining with the albumen to form a harder compound in a manner comparable to the process of tanning, and therefore the choice of a suitable re-agent in each case will be found, in spite of anything said to the contrary, to be a matter of considerable moment, and its mode of use equally important.

Enormous labour has been incurred by one generation of investigators in correcting the errors of a previous generation, who

have dogmatised upon observations of tissues profoundly altered in course of preparation, and all the science of modern histology, with its innumerable precautions and warnings, is one vast machinery for avoiding similar errors in the future.

When a tissue dies, its elements undergo change if left to themselves and in their mutual relations. The cells shrink, fibres swell, objects become effaced, and others unduly emphasised ; hence the precaution of "fixing," which is the first great step in microscopy. Observations made on a soft tissue which has been left untouched for twenty-four hours are mostly worthless. If, for instance, a portion of the retina be left in water after removal from the body, after a few minutes changes commence, the rods and cones disintegrate, nerve-fibres become varicose and appear to be covered with little lumps. If a fixing agent be employed, these elements remain unchanged for any length of time.

Dehydration is another precaution that experience has shown to be necessary, the presence of water tending to assist the post-mortem decomposition.

It is well to remember that it is dangerous to generalise or deduce theories from the observations of objects that have been submitted to a great variety of re-agents in the course of their preparation. For instance, if a section has been exposed to repeated extremes of temperature and raised a number of times to the boiling point—possibly frozen as well—soaked in baths of spirit, subjected to the action of acids, and otherwise tampered with, the facts deduced from the examination of such a specimen are always open to the suspicion of having been created in the process of preparation. The simpler, therefore, the treatment has been, provided that post-mortem putrefactive changes have been duly guarded against, the more reliable will be the observations based thereon.

In Fixing, the action of the agent is, as we have said, to coagulate the albuminoids, gelatin, and mucin present in the tissue. The action of the agent varies. Thus, chromic acid, osmic acid, and chloride of gold chemically combine with the tissues ; and if you use them, you must subsequently stain with hæmatoxylin or an anilin dye, except in the case of gold, which has

some action on the tissues at present unknown, but which renders after-staining useless and impossible in most instances. Corrosive sublimate and picric acid can be washed out, and you may use any stain afterwards.

If anyone will take the trouble to examine the literature on the subject of hardening, they will be greatly surprised at the diversity of opinion among original workers. It should be remembered that most of our text-books are simply re-copies of the older works, and which, coming to the front now that microscopy is more studied, are often looked upon as new methods. It seems to me that if we would consider hardening agents, we should take one re-agent at a time and try hardening every part of the body in it; then make sections, stain, mount, and study the changes by comparison, and having taken one re-agent as a standard we ought to be able to come to some definite conclusions as to their relative manner of acting on the tissues.

One authority says :—"We should never use weak and increasing grades of alcohol to harden with, but always use absolute alcohol." I do not doubt but that if we could afford the expense and be certain of our agent, we should never use anything but absolute alcohol, and then the purest sample known, and never the diluted variety or proof spirit, as the latter, in addition to its admixture with water, is almost always contaminated with other substances, and often exhibits an acid reaction. If we desire to use diluted alcohol, we should mix the absolute variety with the requisite quantity of distilled water to obtain a reliable result. Personally, I have never had any occasion to find fault with the ordinary alcohol; and, except in cases of celloidin and other embedding agents, and to demonstrate Heidenhain's experiments on epithelium, etc., I never have had to use absolute alcohol in micro work, and I do not think it is necessary.

It seems to me that if we are going to consider alcohol of any grade as a *first-class* hardening agent, it ought to stand a fair test—namely, that of a general hardening agent, but *particularly* should it be tested with the most delicate structures, for we can then easily appreciate the sudden changes which will take place in such tissues compared to those which are more resistant. We usually look on the nervous system and the eye as being the most delicate parts of

the body, but personally I think the developing pulp in teeth should be included, for if anything it is more easily changed than the brain.

It is well known that alcohol cannot—or, rather, should not—be used for central nervous-system hardening, and least of all should it be used for the white substance, as it fails to harden properly. Moreover, a large share of the fatty constituents of the medullary substance is extracted by the alcohol, to be precipitated later in a crystalline form, and so injure the tissues. Thin tissues and membranes are also best hardened by some other fluid on account of their curling up. Intestines and tracheæ are good examples of this.

Let us briefly consider the question of using absolute alcohol, as ordered by some. First, remember we must only use the very thinnest and smallest pieces of tissue, and therefore must guard against curling up. Second, we know the moment the alcohol touches the specimen its action is to coagulate; in fact, so quickly does it act, that only the external layer of cells is affected, and the deeper ones are not hardened at all. Why is this? Because we have placed a coagulated mass first, and this acts as a barrier, if I might so term it, and prevents the agent penetrating. This has been proved in the case of bichloride of mercury and its action as a germicide, by its forming a capsule around the bacilli which the agent cannot penetrate, and if the germs are allowed to recover themselves, a chemical action is probably set up which will dissolve the coagulated capsule and set at liberty the bacilli to resume their work.

We know the action of carbolic acid, so let us take it as an example from dental surgery; but unfortunately it is not considered as it should be, for if we use the pure acid we produce a caustic action, an eschar results, and the drug scarcely penetrates except to the first layer of tissue. Try it on the skin. We get no action unless we remove the eschar and again apply, and so on. But let us use a solution of a low strength, and we can get it to penetrate to such a degree as to injure the deeper layers. In a tooth it will penetrate in many cases, irritate the pulp, and often cause its death.

From these facts it will be sufficient to show the restriction

which can be laid on the use of strong alcohol. I have seen many anatomical and pathological specimens which have been preserved (?) in it, and on cutting them open after a year or two the odour is very unpleasant, and shows decomposition, if not putrefaction, to be present. Again. Sarcoma of the large-celled, and even the spindle-celled variety may be readily mistaken for the small round-celled, through the contraction produced. I think in Pathology too much care cannot be taken in the examination of alcoholic-hardened specimens. Always use a weak grade to begin with, unless for some special reasons ; but remember to take the changes into account.

Alcohol should only be used in cases of rapid diagnosis in such cases as require a report for immediate operation ; always, where possible, harden some of the specimen in one, if not in two other agents as well. It can be used for Bacilli in tissues if structural changes are not to be regarded, and in some micro-chemical reactions, possibly amyloid reaction. When distilled water is used to examine these alcoholic specimens, the contraction is got rid of to some extent by the tissues taking up the usual amount of water, but this cannot get rid of the loss of transparency due to the granular coagulated albuminates which will persist. Glycerine as a clearing agent will aid this in some cases, or the acids and alkalis, which dissolve the precipitated albuminates, though only at the expense of many other structures.

If fat is present, alcohol cannot be used. Lungs and muscles do not harden well in alcohol, and sections kept in this country for any length of time become very hard and brittle, and will not stain well. Alcohol is best suited for hardening bacilli tissue, salivary glands, and pancreas.

According to our knowledge of hardening agents, I regard their value in the following order :—First—Müller's fluid and a modification known as Erlicki's fluid ; Second—Chromic Acid ; Third—Flemming's solution ; Fourth—Rabl's fluid ; Fifth—Perenyi's ; Sixth—Bichloride of Mercury ; Seventh—Kleinenberg's solution ; and lastly—Alcohol.

Muller's Fluid—as is well known—is composed of:—

Bichromate of Potassium	2 parts.
Sulphate of Sodium	1 „
Water	100 „

It is the most useful, especially in the preparation of delicate tissues, in which it fixes the protoplasm of the cells rather than hardens them, and in this way causes but little shrinking of the tissues, so that for congested organs, or mucoid tissues, it is invaluable, as also it is for the central nervous system, intestines, and hollow organs. The great advantages of this agent are its liability to harden large specimens thoroughly, even to whole brains; its power to penetrate, caused through the agency of the sulphate of sodium, which, if it once gets into the tissue, the bichromate of potassium can follow. There is, as a rule, no danger of over-hardening, and even if the process takes a considerable time the results are almost invariably satisfactory. For delicate tissues it is unequalled, and it is an essential factor in the special staining processes of Weigert and Pal.

It is said by some authorities (Wynter and Wethered, also by Wethered) that it must not be used for micro-organisms in tissues, nor for micro-chemical reactions as amyloid. I have examined large quantities of material for both these conditions, and thus far have experienced no difficulty; Woodhead, Klein, or any other author do not raise any such objections so far as I can learn.

There is another exception. A deposit of lime salts is dissolved by this medium, and consequently chalky deposits, such as occasionally occur in the glomeruli of the kidneys, are destroyed if the organs be hardened in this way (Wethered), and also the arterial walls are destroyed by chromic salts and thus escape detection. I have hardened basilar, radial, and temporal arteries, heart, aorta, etc., by Müller and in chromic acid, in a case of *Arteritis Deformans*, and in calcific degenerations from other parts, and found no difference.

The disadvantages of Müller's fluid are:—The colour given to the specimen (but this can be removed by chloral hydrate), its length of time to harden, a slight danger to grow mould or fungus, which thus far I have never been troubled with, and which can be avoided by using a little camphor, thymol, or naphthalin, and by

keeping well covered and air-tight. Hardening in Müller can be hastened by raising the temperature to 30 or 40°C. (80–100°F). It should be changed the first, second, and fourth days, and as often afterwards as it is discoloured, and then left alone except for an occasional stir. A large quantity of fluid should always be used. The spinal cord of a dog or cat requires about a litre of the agent. Harden in a dark, cool place. If a quicker solution is required, use Müller's fluid and spirit, three parts to one.

If a Brain is being hardened, inject it daily with the fluid, keep it for four or five days in a jar of the same fluid, change daily, and then it can be cut up as desired. To hasten the hardening of a brain or spinal cord that has been in Müller's fluid for three or four weeks, a two per cent. solution of bichromate of ammonia will do it very nicely in about fourteen days if the fluid is changed daily. These agents are particularly good for nerve-tissues, brain, spinal cord, retina, intestinal muscle, and glands.

Erlicki's Fluid is another good agent, and is very similar to Müller, but contains half per cent. of sulphate of copper, instead of one per cent. of sulphate of soda; it is quicker in its action, and this can be hastened if the tissues are kept for a day or two in it at a temperature of 40°C. Two days or so are sufficient under these conditions.

Chromic Acid.—This is best when used as a one-sixth per cent. chromic acid. Take of it two parts, and spirit one part, and stir. Cut the tissue in pieces not larger than one-fourth of an in. square, and put them in a quantity of the fluid. First day, put the tissue in the fluid. On the second, fifth, and eighth days, change the fluid. On the ninth day, put the tissue in a weak spirit mixture (two parts alcohol and one water; stir). Tenth day, remove to pure spirit. On the fourteenth day, put it in plain water. On the fifteenth, remove to a fairly thick solution of mucilage. On the sixteenth day, put sections into hot distilled water to soak out the gum, then change to alcohol. Stain and mount. The weak spirit mixture is made of two parts spirit and one part water. On the fourteenth day the hardening process is complete, and the specimen may be left in the pure spirit for any length of time, but if it is desired to cut sections the rest of the directions should be followed.

The only objections I know of to this agent are:—Micro-organisms in tissues do not stain after it ; tissues must be cut into small pieces, not more than quarter to half inch cubes, which is not good when the form of a growth or a *macroscopical* specimen is required. Staining must also be done, in most cases, with logwood or aniline dyes, for carmine will not stain well. Tissues get brittle if over-hardened, as they do in all re-agents ; otherwise, it is the most excellent agent we have for all tissues, including even the nervous system, when the tissues may be cut in small pieces.

Flemming's Solution is excellent for embryonic specimens, dental histology, embryology, and mitosis. Its composition is—

Chromic Acid (1 per cent. solution)	...	40 cc.
Osmic Acid (2 per cent. solution)	...	12 cc.
Glacial Acetic Acid	3 cc.

Pieces of tissue must be very thin, not more than two to three millimetres in size. They will harden in from ten to twelve hours, and after being hardened must be washed in *running water* for hours, and then finished in alcohol of various grades from weak to strong.

Fol's Solution is well known and is very similar, but has less osmic acid, and is used more generally on account of less expense.

Osmic Acid (1 per cent. solution)	...	2 cc.
Chromic Acid (1 per cent. solution)	...	25 cc.
Glacial Acetic Acid (2 per cent. solution)	...	5 cc.
Distilled Water	68 cc.

For both Flemming's and Fol's solutions materials must be cut and stained immediately, as after keeping they do not stain well. Saffranin and logwood are the best staining agents for these methods.

Rabl's Fluid is made by taking

Chromic acid (1/3rd per cent. solution)	...	200 cc.
Formic Acid	4 or 5 drops.

It must always be freshly prepared, the tissues must be fresh, and cut in small pieces. Harden for from twelve to twenty-four hours ; then wash thoroughly in distilled water, and finish in various grades of alcohol. Stain with hæmatoxylin or saffranin. It is especially valuable for mitosis and nuclei generally. Its advantage is that tissues hardened in it do not afterwards darken.

Pacini's Fluid.—

Bichloride of Mercury	1 part.
Common Salt	2—4 parts.
Water	200 parts.

When 2 parts of salt are used, this formula is very useful as a general preservative, but especially is it useful for blood-corpuscles of cold-blooded animals, as it has a less density than the formula with four parts of salt, which is better for the blood of warm-blooded animals. It preserves spermatie fluid, epithelial cells, nerves, muscle fibres, and fixes infusoria by adding a small quantity to the water containing them.

Pacini's Preservative Fluid for all delicate tissues is made by

Corrosive Sublimate	1 part.
Sodium Chloride	2 parts.
Glycerine (25 per cent. Baumé)	13 parts.
Water	113 parts.

Allow the mixture to stand undisturbed for at least two months. Then take for use one part, mix with three parts of water, and filter.

Perenyi's Fluid.—

Nitric Acid (10 per cent. solution)	...	4 parts.
Chromic Acid (0.5 per cent. solution)	...	3 parts.
Alcohol	...	3 parts.

Mix. Immerse the specimens for four to five hours; then use gradual strengths of alcohol, 70 per cent. for twenty-four hours; strong alcohol for several days; then absolute alcohol for four to five days; then cut. The advantages are that segmentation spheres and nuclei are fixed perfectly, ova do not get porous, but cut well, and the stain can be combined with the fixative. In the case of ova, the albuminous envelope must be removed. It is also excellent for dental histology, particularly development.

Picro-sulphuric Acid—either Kleinenberg's or, better, Mayer's formula—are very good, the latter saving the waste of the picric acid. Always use a large quantity of the solution, and change as often as it is cloudy. It hardens very rapidly (four hours is usually enough), and then wash in 70 per cent. alcohol; warm alcohol extracts the acid more quickly than cold. Remember *never* to

wash out picric acid or bichloride of mercury with water ; always use alcohol.

The picro-sulphuric acid has great penetrating powers, and is totally soaked out of the structure with alcohol, leaving them in a good condition to stain. If much lime is in the tissues it is not to be recommended, for it dissolves out the lime and throws it down as crystals of gypsum in the tissues. For such the picro-nitric or picro-hydrochloric acid is to be preferred (Mayer's formula).

Bichloride of Mercury.—The other agent is a most rapid hardening one, so that tissues must not be left in it for too long a time. For small pieces, a quarter of an hour or thereabouts is sufficient ; for large pieces, one to two hours. The pieces when fixed become whitish throughout.

For glands and glandular structures generally, a half-saturated alcoholic solution is most useful—*i.e.*, to 50 cc. of a saturated alcoholic solution add 50 cc. of 70 per cent. alcohol. Vignal recommends that to 100 cc. of this mixture there be added five to six drops of nitric acid. The pieces of glands should be cut into cubes about four millimetres in size, and can be hardened in an hour or so. Finish in alcohol. I object to this agent as a general one, for it contracts so much, and it does not bring out the finer structural details, as the striæ in the muscles of the tongue, etc.

A saturated watery solution contains about 5 per cent. of this salt ; but it is much more soluble in alcohol, especially alcohol of 50 to 60 per cent. Keep both a saturated watery and a saturated alcoholic solution on hand. The salt will hasten hardening if the fluid is heated to 30° C., and all stains can readily be used after it. Specimens, unfortunately, after bichloride hardening, if left in alcohol for any length of time, become brittle. It is best to stain and embed in paraffin, where they can be kept for some time, either dry or in alcohol.

Dilute Nitric Acid (such as Altmann's method) is very useful and applicable to most delicate objects in histology, even to the retina. It is claimed to be the most trustworthy fixing agent for protoplasm, but if so, why is it not more used ? So far, I believe it is chiefly used in embryology by a few special investigators. A secondary point is that it is one of the best dissociating and decalcifying agents.

To Harden in Alcohol.—Use on the first day a mixture of two parts of alcohol in one of water ; stir. Then for four days harden in ordinary alcohol, and then proceed to cut, etc., as given on page 376, in the chromic-acid method.

In conclusion, I will briefly mention the points to be attended to in order to secure good results by hardening :—

1.—Cut the specimens with a sharp knife so as to get a clean cut.

2.—All tissue must be small except for Müller's fluid.

3.—Do not let the tissue touch the sides or bottom of the jar unless cotton-wool, or tow is between.

4.—Fill the jar with a quantity of fluid (20 to 100 times the bulk of the tissue).

5.—Label carefully with the name, age, sex, organ, disease, date, and time of hardening and agent used.

6.—Keep in a cool, dark place, especially all the chromium salts when hardening, and no deposits will occur.

7.—Change the fluid as often as it becomes discoloured, and always during the first day or two wash the jars, to rinse them of deposits, blood, etc.

8.—Do not over-harden. Always test by feeling to ascertain the consistency ; never allow tissues to get brittle.

9.—Harden slowly ; wash all colouring matter away by *running* and not still water, but do not allow the tissues to become sodden.

10.—Always use a weak spirit mixture, made of two parts spirit to one of water, before ordinary spirit is used.

11.—Change any spirit which becomes cloudy.

12.—Pure chromic acid solutions do not penetrate ; hence, alcoholic solutions are best, as the alcohol aids in penetration. Chromic acid seems to form a compound with the tissues, so that it is not easily removed from them. Carmine will not readily stain these tissues, but hæmatoxylin, etc., will.

13.—Tissues should always be as fresh as possible, and the solutions also.

14.—Much blood, etc., may be washed off by normal salt solution or by some of the hardening agent first.

15.—Picric acid and alcoholic bichloride hardened tissues must not be washed or placed in water, or the sections after the bichlo-

ride will be dotted with small black specks or star-like crystals of the salt. They should be washed directly in various strengths of alcohol. In the picric acid tissues, water swells and injures the specimens.

16.—Use several hardening agents on one tissue and test the results. No definite instructions can be given for individual cases, but the following general rules will assist in determining what should be the nature of the hardening fluid:—

(a) Where a tissue is hard and firm, and not likely to shrivel on the abstraction of water, and where, too, it is not thought necessary to keep blood in the organ, spirit may be used.

(b) Where there is much blood in the tissue to be hardened, or where the tissue is very soft or œdematous, use Müller.

(c) For small objects of very delicate structure, use osmic acid, Rabl's, Flemming's, Kleinenberg's, chromic acid, or Müller.

(d) If bacilli or bacteria are suspected, use alcohol, weak or absolute. In some cases, however, a previous treatment with Müller's fluid is a great advantage.

If these directions are carefully followed, good results will be obtained, and for successful pathological and histological investigation so much depends on this preliminary work—(in spite of its being said, "We have such perfect methods for embedding it is not necessary")—that I advise all to pay every attention to them, even to the minutest detail.

ORIGIN OF ATMOSPHERIC OXYGEN.—Dr. T. L. Phipson, who has devoted a considerable amount of attention to problems concerning the constitution of the atmosphere, is led to the conclusion that the original atmosphere of the globe consisted of nitrogen alone, and that the oxygen now present is the product of vegetable life. In a paper in the *Chemical News*, he states that minute microscopic plants (*Protococcus pluvialis* and *P. palustris*) can be easily transformed into manufacturers of oxygen gas. As the results of experiments, he concludes that plants absorb carbonic acid gas by the roots and secrete oxygen by the leaves, from which it is subsequently given off. Into the primitive atmosphere of nitrogen the early vegetation would thus pour oxygen during countless years, until its composition became practically what it is now.—*Pharmaceutical Journal*.

***Synchaeta tavina* (sp. novo).**

By JOHN HOOD, F.R.M.S. Plate XVII.

IN March and April of this year (1893), I trawled out of the waters of the Firth of Tay in great numbers a large rotifer resembling in outward form the fresh-water species, *Synchaeta tremula*, Ehrn., but which was much larger in size. It not only differs in the nature of its habitat and in bodily dimensions, but there is also a slight difference in the arrangement of the frontal ciliary wreath; the coronal cilia are arranged in cushion-like tufts, very suggestive of the ciliary arrangement of *Hydatina senta*, with the addition of a pair of small ciliated auricles, one on each side; these auricles are present to a greater or less degree in each species of the genus *Synchaeta*.

There are now eight or nine species of the genus known to scientists. Seven of these have their corona formed in a more or less convex or rounded shape. But *S. tremula* and the new marine species—which I propose to call *Synchaeta tavina*, after *Tavus*, the classical name of the river Tay—have their corona truncated, or flat across the frontal head, and each are furnished with four long rigid bristles or styles, set nearly equi-distant on the coronal head. Their function is probably that of feelers or organs of touch.

The chief characteristic and specific difference between other species of *Synchaeta* and the *S. tavina* is that the latter has two occipital eyes, while all the other eight species possess one eye only. The two eyes in *S. tavina* are set close together; they are of a dark reddish colour, situated on the lower lobe of the occipital nervous ganglion or brain.

The eyes are large and are quite readily observed from either a dorsal, ventral, or lateral view. A nerve-thread leads from the base of the brain to the dorsal antennæ, situated in the neck. There are a pair of γ -shaped gastric glands at the top of the stomach—one on each side of the œsophagus—which is peculiar to *S. tavina*, whilst in all the other species the gastric glands are round.

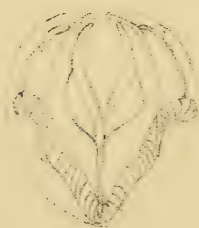
The Mastax and the arrangement of the other internal organs



Dorsal view.



Side view.



Jaws.

are similar to the other species of the genus. I noticed that the stomach of all the specimens I have examined were packed full of a brownish matter. *S. tavina* is a vigorous swimmer. Its motions are zigzag or in spiral circles. I have never witnessed it indulging in spinning round on its own axis and occasionally remaining steady in the field of view, as is the habit of its fresh-water sister, *S. tremula*, but it always appeared dashing on at a rapid speed.

I have never observed a single specimen carrying its eggs at its foot—a practice which I have frequently observed in *S. tremula*.

It is to me a very remarkable fact that I have only found this creature in any considerable numbers at high water on spring tides; and although I have repeatedly trawled for it at high water at neap tides, I have seldom found a half-dozen specimens. But when spring tides came round, during March and April, it was found in great abundance. This phenomenon I cannot explain, except it be that, like the herring, *S. tavina* always keeps in shoals.

Occasionally I have found it associated with *Synchæta baltica*, Ehrn.; *Pterodina clypeata*, Ehr.; *Distemma raptor*, Gosse; *Diglena suila*, Gosse; and invariably with *Notholca thelassica*, Gosse; *Notholca schapha*, Gosse; *Notholca Hoodii*, Western; and with varieties of Entomostracans, Infusoria, and Marine Diatoms of various forms, most especially the *Pleurosigma angulatum* and *P. elongatum*; while the delicate *Amphiprora constricta* was always present in the sediment.

The male has not yet been observed. A few females were observed with ephippial eggs within their body-cavity, which clearly indicated that the male had been present a short time previously, but had escaped observation.

The length of fully-developed adults is from one-seventieth to one-eightieth of an inch.

EXPLANATION OF PLATE XVII.

- Fig. 1.—*Synchæta tavina*, side view.
,, 2.—The same, dorsal view.
,, 3.—Jaws of *Synchæta tavina*.

A Cheap and Efficient Dissecting Microscope

WE think the annexed illustration of this instrument, Fig. 84, will explain itself. The stand is made out of a solid block of clear pine, as shown in *A*, front view ; *B*, end view ; *C*, median cross section, showing *m*, an inclined mirror, fastened with small screws or tacks. *D*, top view ; *l.h.*, lens holder, which slides in brass tube driven into a hole in block (see *C*) ; *st.*, stage, a moveable glass plate.

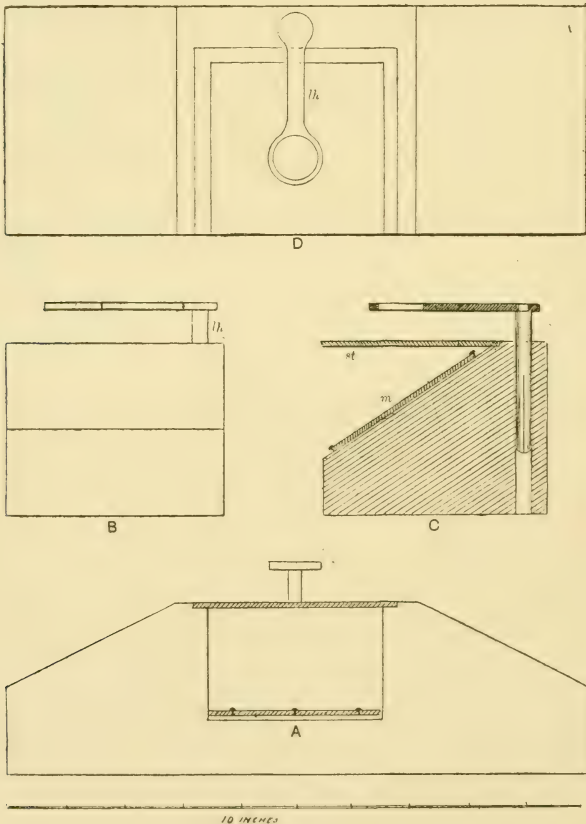


FIG. 84.

Notes on the Breeding Habits and Embryology of Frogs.*

BY J. H. MORGAN.

THE following notes are the outcome of several years' observations on the breeding habits and stages in the development of frogs. They are confessedly very incomplete, having been collected rather as an amusement than with any desire to increase our present knowledge of amphibian embryology. Some older observations have been verified, and I believe a few new observations made which perhaps are worth recording. From many points of view I think the development of the frog is better adapted to the need of students beginning the study of embryology than the classical chick. Certainly this seems to be true if a clearer knowledge of the phenomena of development in general is desired, and not merely an introduction to human embryology—the best excuse offered for presenting the hen's egg and chick, with its mistifying yolk and white, and its incomprehensible (to beginners) larval membranes. On the other hand, the ease with which the young chicks are to be obtained at all seasons, makes a very strong argument in their favour. Correspondingly, the difficulties of removing the younger stages of the frog's egg from the surrounding jelly has been a great drawback to the study. Appreciating this last difficulty I have experimented, for several years, on methods of removing these jelly membranes. At last I think I have successfully solved the problem, and can now obtain, with the greatest ease, the most difficult stages, which are also in perfect histological condition. The method will be given in Section 9. Clay models of the early stages of segmentation of the egg have been a very useful addition in presenting to others the arrangement of the cells. These, of course, should be copied from actual eggs, and not from the perfectly regular (but entirely schematic) figures of the ordinary text-books.

The following subjects are touched upon:—(1) Time in laying, and Localities; (2) Laying in confinement; (3) Polar Bodies; (4) Segmentation of the Eggs; (5) Orientation of the Egg; (6) En-

* From *American Naturalist*, 1891.

closure of the Light Pole by the Dark Pole ; (7) Effect of Temperature ; (8) Time of Hatching, etc. ; (9) Methods of Technique.

I.—Time of Laying, and Localities.—The observations were made in the vicinity of Baltimore, Md., during the spring months of the years '88, '89, '90, '91. I shall only speak of those species of whose identity I am certain. Other and more imperfect observations are left out. The first frogs to lay, and amongst the very first (*Acris gryllus* excepted) to appear, are the wood-frogs, *Rana Sylvatica*. A few warm days in early spring time suffice to bring them out. The following records give a general idea as to the time:—February 23rd, '91, and March 8th, 9th, and 10th, '90. The eggs of these had been laid several days. The egg bunches are found in small pools on the edges of woods, generally among the low hills, and are often stuck to twigs of bushes. The bunches are generally large, four to six inches in diameter, and contain very many good sized eggs. In the same pool it is quite usual to find the firmer egg-bunches of *Amblystoma*, as this Urodele also lays its eggs very early.

Somewhat later, two species of tree-frogs appear in the small pools in the woods, generally in quite small, and therefore, during the daytime, often quite warm, puddles ; sometimes in the same pools as the wood-frogs, oftener in the ditches by the side of the road. These tree-frogs are *Hyla Pickeringii* and *Chorophilus triseriatus*. They are often found paired, and may be, in this condition, carried to the laboratory, where they continue to lay for hours without abatement. The eggs of these species are very similar, and I know no certain method of distinguishing one from the other. The bunches are small, attached to bits of grass, or lie simply at the bottom, and each bunch contains from five or six to fifteen or twenty eggs. I have the following record of times at which the eggs were found : *Hyla*—March 9th, 10th, 13th, April 5th, '90 ; *Chorophilus*—February 23rd, '91, and March 13th and 24th, '90.

The eggs of *Rana halcina* are found still later, sometimes in the same localities as the wood-frogs, often in pools in the open ground quite away from the woods. The eggs are individually smaller, so that, although the jelly masses are often as large as those of the wood-frogs, the number of eggs is greater. The fol-

lowing are the records : March 5th, April 5th, '90. The eggs of *Rana clamitans* are not so certainly referred to as its adult, and I have only strong probability showing them to belong to that species. The bunches much resemble those of *Rana halecina*, but the eggs are larger and the jelly firmer. Those I have found were also attached to twigs of bushes, which is not always the case in *Rana halecina*.

The toad (*Bufo lentiginosus*) in this latitude lays very late in the spring. The eggs are easily distinguished from the frog's, as they are laid in long strings, often yards in length, the eggs arranged (generally) in a single row. They were recorded April 14th, 1890, April 5th and 6th, 1891. The best localities seem to be those parts of rivers or streams where the water backs up, and to one side protected by a bar, so that the eggs are not carried away by the water, and where the water itself is often exceedingly warm. Copulating individuals are easily obtained, and they continue to lay in confinement.

II.—Laying in Confinement.—If frogs are caught at the height of the breeding season, they can often be got to lay in confinement. The surest way is to get the paired individuals, frightening them as little as possible, and placing them in dishes or aquaria containing the requisite amount of water. Only once have I had the wood-frog lay in the laboratory, although with proper precaution there seem to be no very great difficulties of obtaining in this way the eggs of the species. A single large bunch of eggs were laid by this pair during the night, which developed normally.

By far the best and easiest eggs to be obtained, by bringing frogs into the laboratory, are those of the tree-frog named above. They will continue to lay small bunches of eggs for as much as twenty-four hours after catching them. By removing the bunches as fast as laid, an exact record may be kept as to the age of the different lots. Moreover, the eggs of these species are small, and the jelly clear, so that they are well adapted for study of the segmentation stages under the microscope. The distinction between the cells derived from the black (animal) pole, and those from the yellow (vegetative) pole, is very sharp, and the fate of the cells more easily traced through the later stages of segmentation. Toads brought into the laboratory, and placed under proper

conditions, continue to lay for many hours. A single copulating pair, which were laying eggs when captured, were isolated *over night* from other individuals, and, in the morning, a long string of eggs were found. Dr. E. A. Andrews carefully estimated the number of these, and found that, inside of ten hours, the female had laid the astonishing number of 28,000 eggs, and the male had fertilised them. This was at the rate of forty-one eggs per minute for ten hours. After the eggs are laid, the male and female separate, and, where formerly they remained quietly in the dishes or aquaria, they now proceed to climb out, and show a tendency to wander over the building.

III.—Polar Bodies.—I have seen these extruded in the egg of the tree-frog. They are found at or near the apex of the black pole, and appear as white spots with a black periphery. Sometimes they are quite near to each other. Again, I have seen them separated by quite a wide distance. They were extruded about one hour after the eggs were laid, as nearly as could be calculated.

IV.—Segmentation of the Eggs.—The series of diagrams ordinarily found in text books on embryology are exceedingly diagrammatic, and give an entirely erroneous impression as to the appearance of the segmenting egg, especially during the later stages. I found this to be the case in the eggs of the tree-frogs (see above) and the common toad, and expected to find a parallel case in *Rana temporaria*—that studied by Ecker, and from whom the text-book figures are taken. During the present spring ('91), I have procured the early stages of segmentation of this frog, and found it to agree in every particular with other species, and therefore to depart from the classical type. Rauber has given excellent figures of the later stages of the frog's eggs, and in many points I have verified his account. The first furrow divides the egg into two equal halves. The second, at right angles to this, gives four equal segments. The third furrow is not equatorial, but lies nearest the dark pole of the egg, the result being in four equal dark cells, and four larger, but equal, light cells. At the next stage, the marked regularity of the preceding stages is lost, and each of the light cells divides, as it were, independently of the rest. The text-book figure at this sixteen-celled stage may be taken to repre-

sent an ideal to which the egg never attains. The division of the sixteen cells into thirty-two does not conform to any rule, although again, but in a less degree, Ecker's figures may be taken to represent, in the most diagrammatic way possible, the planes of cleavage. Without figures it is impossible to describe the precise method of segmentation; those of Rauber approximate, I believe, most nearly the truth. In general, we may say that, up to the eight-celled stage, the segmentation is very regular, but that after that, no particular plane of division can be prophesied for any segment. Often, during the sixteen-celled stage, the upper eight (black) cells are arranged in almost perfect bilateral symmetry, and not a radial one, as given by Ecker.

V.—Orientation of the Egg.—The relation of the first plane of segmentation to the adult has attracted a great deal of interest during recent years. The relation found in the frog's egg has been already studied with varying results. Newport's experiments in 1851, '53, '54, are, I think, the most to be relied upon, and, during the present spring, I have had the pleasure of verifying his results on a small scale. The eggs of the tree-frog were used in the experiment. The outer layers of the jelly were removed from an egg which had not yet divided, or had only undergone the first cleavage. A small triangular piece of card-board was then cut out, and a drop of collodion placed on it. The egg, with its thin layer of surrounding jelly, was placed on the drop of collodion as soon as the latter began to stiffen, and card-board and egg were then immersed in a dish of water. With a pencil, a line was drawn on the card-board, corresponding to the plane of first division. The water was changed several times, until all trace of ether was gone, and afterwards set aside in a quiet and warm place. Several other eggs were prepared by the same process.

At the end of forty-eight hours, the medullary folds began to appear, and it was then seen that the plane between these corresponded exactly, in most cases, to the plane indicated on the card-board, and therefore the obvious conclusion is drawn that the first plane of division divides the egg into two parts, corresponding to the right and left halves of the adult body. In a few eggs, the first plane was somewhat to the right or left of the mid-line of the

adult. The embryo begins to rotate in the egg-capsule very soon after the appearance of the medullary folds, so that, unless observations are made at the very first appearance of the folds, the results will be falsified, on account of the rotation of the embryo from its original position. The eggs of the tree-frogs are especially good for experiments such as these, on account of the rapidity with which they develop, decreasing therefore the possibilities of a secondary change in position of the egg after it has come to rest, and its plane of division marked. I think it would be possible, by keeping the eggs in a warm room, to cause them to develop the medullary folds within twenty-four hours after the eggs are laid.

VI.—Enclosure of the Light Pole by the Dark Pole.—In studying a series of eggs from the segmentation period to the formation of the blastopore, the so-called overgrowth or epibolic growth of the black cells has been observed. I am quite sure, however (except in the immediate region on the dorsal side of the blastopore, and later over its whole extent), that the yellow cells disappear from the surface, not by an over-growth of the first-formed black cells, *but by a process of splitting off of cells from the upper corner of the yellow cells themselves.* In other words, there is not a general migration of black cells, but each remains approximately in the position in which it was first formed, and new black cells are continually added at the periphery of the black cap by the splitting off of cells from the upper ends of the yellow cells, so that Balfour's sentence that the disappearance of the yellow cells "is effected by the epiblast growing over the yolk at all points of its circumference," is somewhat misleading. As a corollary to what I have said, it follows, of course, that there is a continuous formation of new pigment taking place at the periphery of the black area *within the new cells that are being formed, and also within the ends of the yellow cells which go to form the new cells in this region.* I have not studied with sufficient care the gradual turning in of the cells around the rim of the blastopore. In one living egg, however, I saw, in the dorsal region of the blastopore, some of the cells forming the floor of the archenteron gradually disappear within the blastopore.

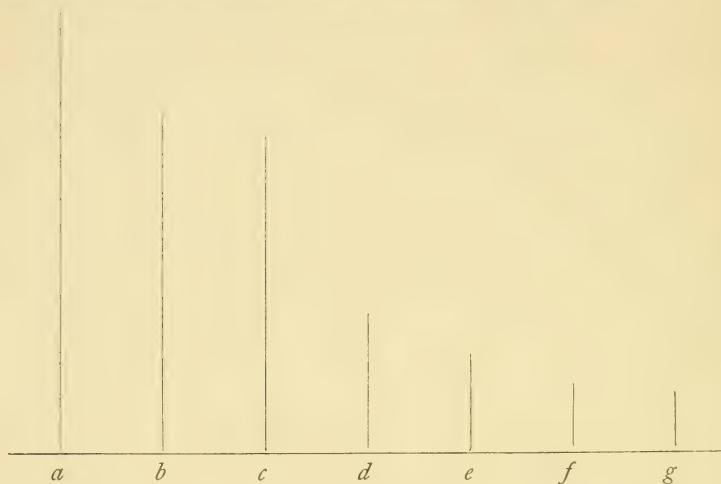
VII.—Effect of Temperature.—It is impossible to give any exact time to the different stages of development, as the time is directly proportional to the temperature of the water within certain limits. The highest temperature is not always the optimum, for several bunches placed in an incubator for hens' eggs were entirely destroyed. The freezing of the water in which the eggs are kept, does not seem to injure the eggs in the least, but simply to retard their development. I have had eggs completely surrounded by ice, and afterward development quite normally. However, when the eggs themselves are actually frozen, they seem to be destroyed, perhaps by the formation of ice spicules within them. The wood-frogs, which lay their eggs so early, generally lose, in this locality, great numbers of them, on account of getting caught in the ice. Those which are not so caught, develop later, when the ice melts, and do not seem, in any way, to be injured by water at the freezing point. I think there is here a most interesting field for experimentation by the physiological embryologist, and I regret I have not kept exact records of the effects of heat and cold.

VIII.—Time of Hatching, etc.—The different species of frogs leave the jelly membrane at different ages. Some have the tail well developed, and are quite active; others have the tail just appearing, and are only able to twist their bodies slowly from side to side, as they cling to the jelly-mass by means of the suckers below the mouth.

The young tadpoles of the wood-frog leave the water as small frogs in the late spring of the same year in which they were laid, that is, become frogs in four to six months. Eggs collected about March 17th, began to change to frogs about June 1st to 14th. These were kept in quite cool water, in a basement room, away from the sun-light.

At the time of transformation into tadpoles, *a sudden decrease in the length of the intestine is brought about.* The tadpoles cease to eat, and the intestine is entirely freed from extraneous matter during this time. The change takes place at the same time that the tail is absorbed within the body (not dropped off, as popularly supposed), and, at the same time, the pair of fore feet, which were enclosed within the branchial fold, break through the exterior. The intestines were removed and measured from the pyloric end

of the stomach to the proximal end of the rectum. Their lengths are recorded in the accompanying table for the wood-frog :—



- (a) From a large tadpole with whole tail and two large posterior feet.
- (b) From a tadpole with whole tail and two large posterior feet.
- (c) From a tadpole with whole tail and two large posterior feet.
- (d) From a young frog, tail beginning to disappear, and four feet.
- (e) From a young frog, $\frac{4}{5}$ tail, and four feet.
- (f) From a young frog, $\frac{1}{2}$ tail, and four feet.
- (g) From a young frog, no tail, and out of water two weeks.

IX.—Methods of Technique.—The eggs during the periods in which it is difficult or impossible to remove the inner jelly membrane, can be freed in the following manner :—With a pair of sharp scissors each egg must be cut out from the general jelly mass, retaining as small an amount of surrounding jelly as possible. It is then put into an alcoholic solution of picric acid for an hour or longer (one to twelve). The solution is prepared by saturating 35 per cent. alcohol with picric acid, and adding the same amount of sulphuric as in Kleinenberg's solution. The solution is not diluted, but used saturated with picric acid. The eggs are then washed for several hours in 35 per cent. alcohol, several hours in 50 per cent. alcohol, and placed in 70 per cent. for several days, changing the alcohol once or twice if necessary. About the second

day the inner membrane begins to swell, due to a slow osmotic action, I think, as the membrane is stretched by tension from within. On the third or fourth day the swollen membrane may be pierced by a sharp needle, and the egg taken out, which is then placed permanently in 80 per cent. alcohol. The method is exceedingly simple, and consists largely in waiting a few days for the osmotic action to take place. Such eggs, if properly prepared, are in excellent histological condition. This simple method has proved so successful that I have not further experimented with it. It is possible that it may be improved by varying the strength of alcohol used, but I have not seen the need of looking further. The membrane does not swell in stronger alcohol than 70 per cent., and weaker would macerate the eggs.

Certain precautions are necessary in embedding the eggs to prevent brittleness. This is obviated by soaking the eggs before embedding for several hours in a solution of turpentine saturated with paraffin, and kept in a warm place—not so hot as the water-bath (50°C.) Heat causes the eggs to become brittle. This is obviated by the above process of soaking, so that the egg need not remain so long as an hour in the melted paraffin of the water-bath. In the younger stages there is no need for very thin sections, but sections 10 μ . thick are sufficient for all purposes. If the sections are cut too thin, the yolk tends to break up and crumble.

STERILISATION OF WATER.—An important communication has been made to the Paris Academy of Medicine concerning the different methods employed to obtain water free from living organisms. This result is very difficult to obtain except by boiling the water, and this plan is objectionable on account of the insipidity of the liquid thus deprived of its dissolved gas. Filters of every description fail to remove the whole of the germs, and, unless carefully and frequently cleaned, may even add to the number naturally existing in the water. After studying the numerous processes in vogue, the investigator decided to adopt a similar plan to that by which suspended minute particles in water are precipitated. The specimen to be purified was first shaken with powdered alum and then left to rest for twenty-four hours. When that time had elapsed the water was perfectly clear, and was also found to be almost completely sterilised. Freshly-prepared sulphate of calcium, oxide of iron, and sulphate of iron are also fairly effective in producing the same result.

Some Further Researches of Prof. E. G. Balbiani on the Merotomy of Ciliated Infusorians.*

BY F. VICENTINI, M.D., Chieti, Italy.

IN a former number (see p. 202) we gave an abstract of the interesting discoveries of Prof. Balbiani on the Merotomy of Ciliated Infusorians; these referred chiefly to *Stentor cæruleus*, and were published in the *Annales de Micrographie* for 1892. In the January number of the same work, for the current year (1893), he followed up the subject by new researches on other species of *Ciliata*, the results of which, together with his views in reference to general cytology, we summarise as follows:—*A*, The Merotomy of *Stentor polymorphus*, *Stentor igneus*, *Dileptus anser*, *Loxodes rostrum*, and *Fabrea salina*; *B*, The Merotomy of *Paramæcium aurelia*, *Paramæcium bursaria*, and *Colpidium*; *C*, Prof. Balbiani's general views in reference to cytology; and *D*, his general conclusions.

A.—The author summarises his experiments on *Stentor polymorphus*, *Stentor igneus*, *Dileptus anser*, *Loxodes rostrum*, and *Fabrea salina*, in the following manner:—

1.—*S. polymorphus* and *S. igneus* exhibit, when divided by merotomy, the same phenomena as does *S. cæruleus* (see p. 202), only those fragments which contain a nucleus are capable of regeneration, whilst those which do not contain a portion of nucleus die in a few days

2.—Some individuals of *S. polymorphus*, which have a normal and healthy appearance, have, at the same time, the nucleus entirely invaded by a parasite (*Holospora obtusa*). Their survival can only be accounted for by supposing that a small portion of nuclear matter still remains in the nucleus.

3.—In *Dileptus anser* (a mono-nuclear species with a diffuse nucleus, and the nuclear matter dispersed in the plasm like minute granules) all the fragments have the power of regeneration, which takes place rapidly (in about four hours), and the regenerated fragments will live for several weeks on the slide like normal individuals.

* *Annales de Micrographie*, Vol. V., 1893, pp. 1—83 (2 pl.).

4.—The rapid regeneration of *D. anser* contrasts very remarkably with the slowness of the same phenomenon as exhibited in *Loxodes rostrum* (a poli-nuclear species). The fragments take as long as four or five days to regenerate into complete individuals as before, but the author is unable to demonstrate that the speed of regeneration bears any close relationship with the mono- or poli-nuclear states.

5.—Sometimes, in the course of regeneration of the fragments of *Loxodes*, a small secondary merozoite, containing one, or perhaps several, nuclei, will form at the expense of the principal merozoite, and in time become free.

6.—*Fabrea salina* (a ciliate of the salt or brackish swamps) regenerates like the fresh-water species. Those fragments which contain no nucleus will probably live for eight or ten days, owing perhaps to the unusual vitality of this species.

B.—The author's researches on *Paramœcium aurelia*, *P. Bursa-ria*, and *Colpidium*, show that—

1.—*Paramœcium aurelia* constitutes an exception with respect to the property of regeneration depending on the nucleus. The mutilated individual will live for a month or more without showing any signs of regeneration.

2.—Only the contractile vesicles regenerate, but their regeneration does not constitute a new organic formation.

3.—When the loss of substance is small, as, for example, a small portion of any extremity of the body, reproduction will only be effected in a series of subsequent generations produced by fission, provided the merozoite lives under most favourable circumstances. In these conditions the regeneration of the posterior pole takes place more rapidly and completely than that of the anterior pole.

4.—Fragments which contain no nucleus regenerate to a much less extent than others containing a nucleus, and disappear more readily in the cultures.

5.—When fragments without a nucleus are stained with sulpho-alizarin-violet, the colour does not change into red or orange in the vacuoles, thus proving the absence of acid. The acid secretion of the vacuoles, and probably also that of other digestive juices, appears to depend upon the nucleus.

6.—The above observation proves that the nucleus can lose the property of regeneration, whilst it retains that of secretion, etc.

7.—The author, at times, observed a remarkable anomaly in the process of multiplication by division, the products of division, instead of becoming free in each generation, remain united, and form an irregular colony by the coalescence of component individuals. Their nuclei, during the process of multiplication, remain united in a knotted mass in the centre of the colony.

8.—The author observes a deformity caused by a prolongation at the margin of the wound, caused by the different layers of plasm, and furnished with cilia, which he considers to be the result of a feeble regenerating power in *Paramæcium*.

9.—In *Paramæcium bursaria*, and in the allied *Colpidium*, regeneration appears to be slow and incomplete, but resistance to regeneration is less than in *P. Auralia*.

C.—References to general cytology or cell-morphology. Physiologically considered experiments in merotomy with respect to the Protozoa are of the greatest importance, in connection with the experiments of Schmitz, Klebs, Korschelt, and Haberlandt, on the vegetal and animal cells.

The first conclusion arrived at through these experiments is that the functions of cells, in reference to the movements of protoplasm (amœboid and ciliary movements, pulsations of the contractile vesicle, etc.) can be accomplished without intervention of the nucleus; while the manifestations of the secretory activity of protoplasm (intercellular digestion, secretion of cellular envelopes, etc., cuticle, cellulose, calcareous, and mucous layers, etc.) depend upon the nucleus.

In vegetal as well as in animal cells, the nucleus rules the cellular secretions, according to the observations of Schmitz and Klebs on the *Algæ*, Hofer and Verworn on the *Rhizopoda*, and the author on the *Ciliata*. Hofer demonstrated the influence of the nucleus on the secretions of the digestive juice among the *Amœbæ*, as did the author in *Paramæcium*. As, by a law of the division of labour, the higher functions of sense and motion belong to the protoplasm, so the functions of nutrition belong to the nucleus.

The function of reproduction appears, according to Prof.

Balbiani, to be the property of the nucleus. In mono-cellular organisms, all the plasms destined to develop into different organs is undivided in the same nucleus, while in the multi-cellular organisms the different plasms are separated and distributed in the various cells destined to form the different elements.

The author sees in the nucleus the chief agent for the re-forming and regenerating activity of the cell. The protoplasm is also active in merotomy, the nucleus and protoplasm appear to act in unison, the protoplasm possessing the power of internal movements which the nucleus rules by co-ordination, according to the form to be assumed by each organ.

The life of the cells, says the author, is not exclusively either in the protoplasm or the nucleus, but results from reciprocal relations of the two elements. But what quantity of nuclear matter, he asks, is necessary to produce regeneration? He concludes that the actual quantity of nucleus makes no difference, and that a single particle acts precisely the same as a much larger quantity. The question then arises, Why are the nuclei of the Ciliata in chains, and those of *Loxodes rostrum* and *Opalina* so numerous? The author supposes the super-abundance is required for certain secretions, as well as for repairing damaged and missing parts, as described in *Dileptus anser* and *Loxodes rostrum*.

Having briefly summarised the work, it only remains for us to add (*D*) the author's general conclusions :—

1.—Among those ciliated infusorians which may be considered as more important for the physiological study of cells, certain functions are accomplished by protoplasm, and others by the united action of the nucleus and protoplasm.

2.—The chief functions of protoplasm are :—(*a*) The different forms of motion, ciliary action, the reception and expulsion of food-particles, pulsation of the contractile vesicle, constriction, and other movements of the body, etc. ; (*b*) the faculty of directing the body in its various movements.

3.—The functions accomplished by the united action of protoplasm and the nucleus are—(*a*) the various cell secretions, e.g., of the cuticle, the acid juice of the alimentary vacuoles, and probably also of the other digestive juices ; (*b*) the regeneration, or re-con-

struction of the organs, and of the general form of the body ; (c) the last stages of the division.

4.—The protoplasm and nucleus exist and work together in harmony, by which the vitality of the organism is maintained, each substance performing its own proper functions.

A New Method of Preparing the Spinal Cord for Microscopical Examination.*

BY EDWIN GOODALL, M.D.Lond., B.S., M.R.C.P.

IN attempting to prepare sections from the fresh spinal cord for microscopical examination, I have hitherto met with disappointment. As far as my knowledge goes, attempts of the kind have always failed. My own experience is that sections can be made from the ether-frozen cord, and floated on water with comparative ease, but that they become quickly spoiled by further manipulation. If they are treated after the method employed for the fresh *cortex cerebri*,† the tissue composing the white matter is thrown into a number of folds, and the sections are thereby rendered useless. But a still graver defect, and one which effectually condemns this procedure when applied to the spinal cord, is the complete absence of fixation of the elements of the white substance. The ordinary appearance of nerve-tubes in transverse section is wanting ; in place of it one sees a tangled skein of connective tissue-fibres and axis cylinders, the latter especially being twisted and corkscrew-shaped. There are literally no nerve-tubes to be seen. As regards the grey matter, the nerve-cells are well stained, and considerably larger than in a hardened specimen, but other tissue elements appear but indifferently.

The following are briefly the steps of the method now adopted :

- 1.—Remove spinal cord from a recently killed animal.
- 2.—Place a portion, 6 to 8 millimetres high, on the ether-freezing microtome ; freeze and cut. The precautions used in freezing the brain cortex to be observed here also.

* From *The British Medical Journal*. † Bevan Lewis.

3.—Float the section on to water. It should be free from wrinkles; if allowed to sink beneath the surface they appear at once.

4.—Take the section up as soon as possible with a perforated lifter, drain off excess of water on blotting-paper, and float the section on pure piridin.* Kept at hand in a watch-glass; it may presently be pushed beneath the surface of the liquid. Hitherto I have kept sections in the piridin for at least one hour—usually several hours. In one case one quarter of an hour sufficed to fix the tissue-elements, so that the time above mentioned is, perhaps, unnecessarily long.

5.—Wash well in water.

6.—Stain.

7.—Dehydrate and clear in piridin (possibly desirable to pass through piridin diluted with water into the pure reagent).

8.—Mount in balsam suitably thinned with piridin.

In this way sections free from wrinkles are obtained. It certainly may occur that out of a large number of sections some present a slight fold at the point of exit of one of the nerve roots, but most obtained are quite free from wrinkle. Such sections are instructive, even unstained, examined simply in water upon the slide. The horns stand out in relief against the darker white substance, and the nerve-roots are clearly seen, sweeping outwards in broad bundles. Within the horns appear large, colourless ganglion cells, and an intricate meshwork of fine nerve-tubes. All these structures approach very nearly the natural size. Such fresh specimens would probably show tract degenerations well under the low power. In stained specimens the transverse sections of the nerve-tubes in the white substance are brought out exceedingly well; the contour of the tubes is sharp; the axis cylinders are well stained. The appearance of the white column is such as one is accustomed to see in sections from hardened cord; the effects of corrugation are, however, absent, and the wealth of structure is greater than in the latter case.

Any of the dyes ordinarily used for the purpose may be employed for staining the axis cylinders and connective tissue of the white matter, but I have mostly used anilin-blue black ($\frac{1}{4}$ per cent.

* First recommended, I believe, by De Souza (*Zeitschr. f. wiss. Mikroskopie*, Bd. v., H. 1) as a hardening reagent for brain substance.

aqua solution). followed by picro-carmin, as I find the combination more effectual than any single stain in bringing out all the structures in the section (grey and white matter). Kernschwarz, diluted to one-third the original strength (five to ten minutes) is an excellent stain for the axis cylinders ; it stains nerve-cells rather too uniformly. By using subsequently the Biondi-Ehrlich stain (ten minutes) they are brought out better. An entirely satisfactory staining of the grey matter is yet to be obtained. By means of Leoffler's alkaline methylene blue (aqua solution, dilute before use with an equal volume of water), the nerve-cells and the connective tissue throughout the section can be stained, but the nerve-tubes are poorly shown. Excess of dye is removed by the piridin which follows. Picro-carmin (half-an-hour) stains all the structures present ; but I have hitherto got the most satisfactory results by staining first in anilin-blue-black of the strength given, for twenty minutes, and subsequently in picro-carmin for fifteen to twenty minutes. The staining of the grey matter will, I hope, be presently improved upon. My immediate object is more especially to bring to notice the fact that by means of piridin, sections of the fresh cord can be fixed and preserved unwrinkled, each nerve-tube being well defined.

Sections prepared and mounted in the manner described show scarcely any diminution in size when compared with the original cord. The rapidity of the method is noteworthy, for by it the cord can be removed, cut, stained, and mounted in the course of a day ; indeed, it is probable that from two to four hours would suffice.

In conclusion, I may state that I have hitherto practised with the cord of the cow, cat, and rabbit respectively, in each case successfully, with the reservation regarding the grey matter alluded to. The longest period which has elapsed between the death of the animal and the time of cutting sections (after which the elements were thoroughly fixed) is an hour and a-half (cow's cord). I have not yet cut any later than this. Judging from certain specimens, in which nerve-roots appear outside the cord in transverse section, I am disposed to think that the same method may be advantageously employed in peripheral nerves.

Simple Apparatus for Gathering Microscopic Objects.

By G. M. HOPKINS.

ONE of the difficulties experienced by a beginner in Microscopy is the finding and gathering of objects for examination.

As a rule, cumbersome apparatus has been used. The conventional apparatus consist of a staff, to which are added a knife, a spoon, a hook, and a net ; but a great deal can be accomplished with far less apparatus than this.

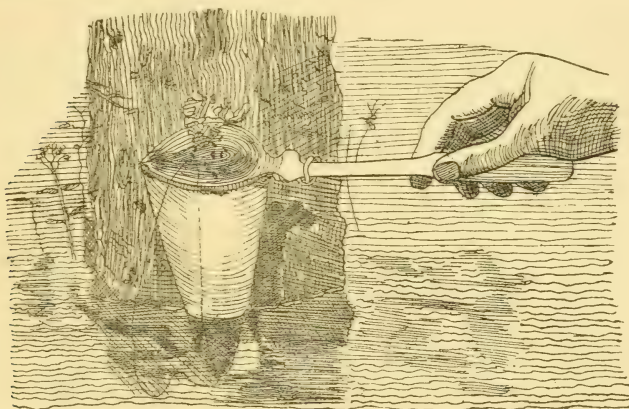


Fig. 85.

The engravings illustrate a simple device, by means of which the amateur microscopist can supply himself with as much material as may be required. It consists of an ordinary tea or dessert spoon, and a wire loop of suitable size, to extend around the bowl of the spoon, having the ends of the wires bent at right angles, and in opposite directions. To the loop is fitted a conical cheese-cloth bag, and to the bottom of the bag, upon the outside, is attached a strong string, which extends over the top and down to the bottom in the inside, where it is again fastened. The spoon is inserted between the bent end of the loop and turned, and the point of the bowl is slipped through the loop.

The instrument is used in the manner shown in Fig. 85 ; that is to say, it is scraped along the surface of objects submerged in the

water, the water passing through the cloth, and the objects being retained by the conical bag. When a quantity of material has accumulated, the bag is turned inside out by pulling the string, and the pointed end of the bag is dipped a number of times in water contained in a wide-mouthed bottle.

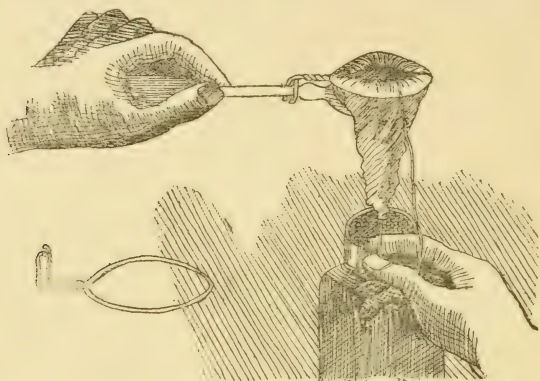


Fig. 86.

The operation of collecting is then repeated. The objects thus washed from the bag are retained in the bottle for examination.

The common method of examining small objects of this kind is to place a drop of water containing some of the objects upon a glass slide by means of a drop-tube, then to apply a cover-glass, and remove the surplus water by the application of a piece of blotting paper. This answers very well for the smaller objects, but larger ones must be examined in a tank or animalculæ trough, which may be obtained at a trifling cost of all opticians. To vary the thickness of the body of water contained in the tank, one or more glass slips are inserted behind the objects.

—*Scientific American.*

WHEN A FOREST has been removed by fire or otherwise, it commonly happens that a fresh growth of entirely new plants immediately springs up. This may be partly due to the unusual opportunity for growth thus given to foreign seeds ; but the usually accepted explanation is that the new growth is from seeds which have long lain dormant.

Drawing with Beale's Reflector.

By G. H. BRYAN, M.A.

THE neutral-tinted glass reflector, or its simpler equivalent, a cover-glass fixed at an angle of 45° with the tube of the microscope, would doubtless be used much more universally for drawing microscopic objects but for its radical defect—that it gives a reversed drawing. This does not matter, as a rule, when the drawing is finished, for, with the exception of spiral structures (which would be changed from right-handed to left-handed screws if drawn reversed), there are few microscopic objects in nature of which a reversed drawing would be an inaccurate representation. The difficulty comes in principally when it is required to fill in the details of the drawing by ordinary freehand, after the outline has been sketched out, for it is impossible to trace the finer markings of objects by means of the reflector, and when the latter is removed, the drawing will be seen to reverse the object whichever way it is placed. How many microscopists have thought of the simple expedient of substituting for the plain glass reflector one silvered at the back, and then copying in the details from the reflected image instead of removing the reflector and looking straight down the tube? A correspondent of *Science Gossip*, some few years back, suggested another plan, which may sometimes prove useful—namely, to lay the slide on the stage cover downwards and sketch the outline with the reflector, and then to turn the slide the right way up and copy in the details—an arrangement only possible, of course, with transparent objects, and with low powers that can be focussed through the thickness of the slide.

If a glass reflector is used for drawing *polariscopic* objects, it must be remembered that the glass itself partially polarises the light which it reflects, so that unless the analyser is turned in the right direction the object will be almost, if not quite, invisible. The great loss of light, which in any case results from such an arrangement, may be entirely obviated by making use of the polarising property of the glass reflector, and dispensing with the analyser altogether. If the glass reflector, instead of being inclined

at 45° to the tube, is placed rather more obliquely—viz., at an angle of about 35° with the axis of the tube, or, what is the same thing, at an inclination of 55° to the face of the eyeglass—the light which it reflects will be completely polarised, so that it will serve the purposes of analyser and drawing reflector combined. The loss of light will thus be reduced to a minimum, and the only modification necessary in the arrangement of the apparatus will be that, in consequence of the increased inclination of the reflector, the tube of the microscope, instead of being placed horizontally, will have to be inclined to the horizon at an angle of about 20° —not altogether a disadvantageous arrangement in some respects.

Stains and Staining.

AFTER Mr. Squire had read his very interesting paper (see p. 282 of our July part), in answer to questions he further said :—

In cutting his sections, the softer vegetable tissues were generally placed between the pieces of carrot in a section holder and were cut in that position. The harder pieces he surrounded with paraffin, and all the animal sections were cut after freezing in gum. Thinner sections could be got by embedding or saturating in paraffin. All those wonderful serial sections obtained at Cambridge had been cut by embedding or saturation in paraffin ; but that took some little time to do, and for ordinary purposes it was a shorter process just to freeze in gum and cut the sections from material prepared in that way.

With regard to a remark as to Schultze's solution being perfectly insatiable for iodine, he was surprised at that ; for, as he had stated, with the strength of the solution he used, only 0·1 of iodine would dissolve in 70 cc. No doubt if it was very much more diluted it would take up more iodine.

All the sections shown were mounted in Canada balsam, dissolved in benzole. He generally ran round them a ring of caoutchouc cement, and found that it was very easy to mount them in that way, and they did not break. As to whether

the hæmatoxylin was prepared from fresh logwood or not, that which he had employed was bought, and therefore he could not say how it had been prepared. He had obtained samples of hæmatoxylin from several different places, and where the crystals were well formed, he had not found the slightest difference in the result. Whether there was any difference in hæmatoxylin prepared from fermented or unfermented logwood, he could not say.

Polyzoa.

By W. G. WHEATCROFT. PLATES XVIII.—XIX.

POLYZOA are members of the sub-kingdom MOLLUSCA.

This sub-kingdom is divided into two great divisions:—

MOLLUSCOIDA and the MOLLUSCA proper. The nervous system of the former of these consists of a single ganglion, or principal pair of ganglia, and there is no circulatory organ, and an imperfect heart. Allman describes the members of the class Polyzoa as follows:—"Alimentary canal suspended in a double-walled sac, from which it may be partially protruded by a process of evagination, and into which it may be again retracted by invagination. Mouth surrounded by a circle or crescent of hollow, ciliated tentacles; animals always forming composite colonies."

All the Polyzoa live in an associated form in colonies, sometimes branched and plant-like, as in the Sea Mat (*Flustra foliacea*) (Plate XVIII., Fig. 1), sometimes encrusting, and very rarely are free. "Each polyzoarium," to quote Prof. Alleyne Nicholson, "consists of an assemblage of distinct but similar zoöids arising by continuous gemmation from a single primordial individual. The colonies thus produced are in very many respects closely similar to those of many of the Hydroid Polypes, with which, indeed, the Polyzoa were for a long time classed.

The Polyzoarium, however, of a Polyzoön differs from the polypidom of a composite hydroid in the general fact that the separate cells of the former do not communicate with one another otherwise than by the continuity of the external integument, whereas the zoöids of the latter are united by an organic connect-

ing medium, or cœnosarc, from which they take their origin." The accompanying sketch of *Flustra foliacea* (one of the Sea Mats, Plate XVIII., Fig. 1) will illustrate the foliaceous polyzoa, *a*, representing a portion of the colony, natural size, and *b*, a fragment magnified to show the cells in which the separate polypides are contained. Fig. 2 is the diagram of a Polyzoön (after Allman) : *a*, Region of the Mouth surrounded by tentacles ; *b*, Alimentary canal ; *c*, Anus ; *d*, Nervous ganglion ; *e*, Investing sac (ectocyst) ; *f*, Testis ; *f'*, Ovary ; *g*, Retractor muscle.

I cannot describe its construction better than by giving the exact words of Professor Allman. "Let us imagine," writes this able naturalist, "an alimentary canal, consisting of œsophagus, stomach, and intestine, to be furnished at its origin with a long, ciliated tentacula, and to have a single nervous ganglion placed upon one side of the œsophagus. Let us now suppose this canal to be bent back upon itself towards the side of the ganglion so as to approximate the termination to the origin. Let us further imagine the digestive tube thus constituted to be suspended in a fluid contained in a membranous sac with two openings—one for the mouth and the other for the vent, the tentacula alone being external to the sac.

"Let us still further suppose the alimentary tube, by means of a system of muscles, to admit of being retracted or protruded according to the will of the animal, the retraction being accompanied by an invagination of the sac, so as partially or entirely to include the oral tentacles within it ; and if to these characters we add the presence of true sexual organs in the form of ovary and testis, occupying some portion of the interior of the sac, and the negative character of the absence of all vestige of a heart, we shall have, perhaps, as correct an idea—apart from all considerations of homology or derivation from an archetype—as can be conveyed of the essential construction of a polyzoön in its simplest and most generalised condition.

"To give, however, more actuality to our ideal Polyzoön, we may bear in mind that the immediately investing sac has the power, in almost every case, of secreting from its external surface a secondary investment, of very various constitution in the different groups : and we may, moreover, conceive of the entire animal

—with its digestive tube, tentacula, ganglion, muscles, generative organs, circumambient fluid, and investing sacs—repeating itself by gemmation, and thus producing one or more precisely similar systems, holding a definite position relatively to one another, while all continue organically united, and we shall then have the actual condition presented by the polyzoa in their fully developed state.”

The vast majority of the Polyzoa are fixed, but this is not universally the case. The two investing sacs of the “cœnecium” of a Polyzoön have been called by Dr. Allman the “endocyst” and the “ectocyst.” The “ectocyst,” or external investment of the cœnecium, is usually a brown, parchmentaceous, probably chitinous, but often highly calcareous membrane; and it is by the “ectocyst” that the cells are formed. In *Cristatella* alone, of the Polyzoa, there is no “ectocyst.”

In many cases the “ectocyst” is provided with peculiar appendages, supposed to be weapons of offence and defence, or organs of prehension called “avicularia” (Fig. 3). The avicularia, or “bird’s head process,” differ a good deal in shape, but consist essentially (to quote the language of Bush) “of a moveable mandible and a cup furnished with a horny beak, with which the point of the mandible is capable of being brought into opposition.” In shape the avicularia often resemble the head of a bird. They keep up a snapping movement, which continues after the death of the general colony. The “endocyst” is always soft, contractile, and membranous.

The mouth, according to Nicholson, conducts by an œsophagus into a dilated stomach. In some cases a pharynx may be present, and in others there is in front of the stomach a muscular proventriculus or gizzard. From the stomach proceeds the intestine, which shortly turns forward to open by a distinct anus close to the mouth. As the nervous ganglion is situated on that side of the mouth towards which the intestine turns in order to reach its termination, the intestine is said to have a “nerved flexure,” and this relation is constant throughout the entire class.

Respiration in the Polyzoa appears to be carried on by the ciliated tentacles, and by the “perigastric space,” which is filled with a clear fluid, containing solid particles in suspension. A kind of circulation is kept up in this “perigastric fluid” by means of

the cilia lining the under surface of the endocyst. Beyond this, there is nothing that can be called a circulation, and there are no distinct circulating organs of any kind. The nervous system in all the polyzoa consists of a single small ganglion (Fig. 2*d*) placed upon one side of the œsophagus, between it and the anal aperture, and is apparently really of a double nature. Besides the single ganglion which belongs to each polypide, there is, in some of the Polyzoa, according to very high authorities, a "colonial nervous system," which unites together the various zoöids forming the colony, and brings them in relation one with another. Some high authorities deny that the so-called "colonial nervous system" is really a nervous nature at all.

So far as is known, the Polyzoa are hermaphrodite, each polypide containing an ovary and testis (Fig. 2, *f'f*). There are no efferent ducts, and the reproductive organs and the products of generation—*i.e.*, the spermatozoa and ova—are discharged into the perigastric space, where fecundation takes place, and the impregnated ova escape by special openings in the "body-wall" by dehiscence of the cell, or in some manner as yet not thoroughly understood. Continuous gemmation occurs in all the Polyzoa, the fresh zoöids thus produced remaining attached to the organism from which they were budded forth, and thus giving rise to a compound growth. I am indebted to Professor Alleyne Nicholson's admirable work for the morphological and physiological account, as well as for the figures Nos. 1, 2, and 3.

The microscope has in this, as in so many other instances, been the chief factor in the elimination of the life-history and physiology to which I have directed your attention. As microscopists, I feel sure you will take a lively interest therein. I have been desirous to study the life-history and development of these minute organisms, and as far as possible to note the changes in their structure and appearance during the countless ages which have elapsed since they first inhabited the seas and some other waters of this globe of ours.

Those small organisms—the fossil Polyzoa belonging to the Primary, Tertiary, and Recent geological periods—have left abundant evidence of their continued existence from Primary times in the various strata. We find remains of them in the Lower

Silurian rocks. Their presence in Cambrian seas is, I believe, not a matter of certainty, although there is something very nearly approaching to proof of their existence even as early as Cambrian times. I have specimens from the Wenloch shales, a group of Upper Silurian rocks. Polyzoa seem to have abounded in Silurian seas. During the Carboniferous period it would seem that they were equally plentiful, and to judge from the specimens before us they appear to have developed not inconsiderably.

The *Fœnestellidæ*, or "Lace Corals," seem to represent the highest, or at least the most beautiful, type of the Polyzoa of the Carboniferous period. There are several specimens of these beautiful organisms in slide No. 2. If you compare Figs. 4 and 5, I think you will agree with me that these little builders had improved very much in their architectural designs since the close of the Silurian period. Whilst those characteristic dwellers in Palæozoic seas, the Trilobites, were fast dying out, their little neighbours, the Polyzoa, were not only holding their own in the struggle for existence, but were developing into forms of greater beauty.

It has been observed by an eminent living naturalist that "in the confederation of animated nature some races can boast of an immemorial antiquity, whilst others are comparative parvenues."

The Polyzoa belong to an ancient race. Some members of this long-lived family have been contemporaries with the Trilobites of Silurian seas, with Pterichthys and other extinct fishes of Devonian times, with the Labyrinthodon and other great reptiles of the Triassic period, with Ichthyosauri, Plesiosauri, and other gigantic saurians of the Jurassic period (specimens of some of which adorn the walls of the Lecture Hall of the Bath Literary and Scientific Institution), as well as with other remarkable types of more recent periods, now also extinct.

It must, however, be assumed that this interesting family of Polyzoa has, like most other families, experienced changes. One branch—the lovely *Fœnestellidæ*—does not appear to have survived the Carboniferous period. We find, however, the family represented by other branches in each succeeding geological period, and we know that it is still well represented in the oceans and seas of the present time by myriads of descendants (Fig. 6).

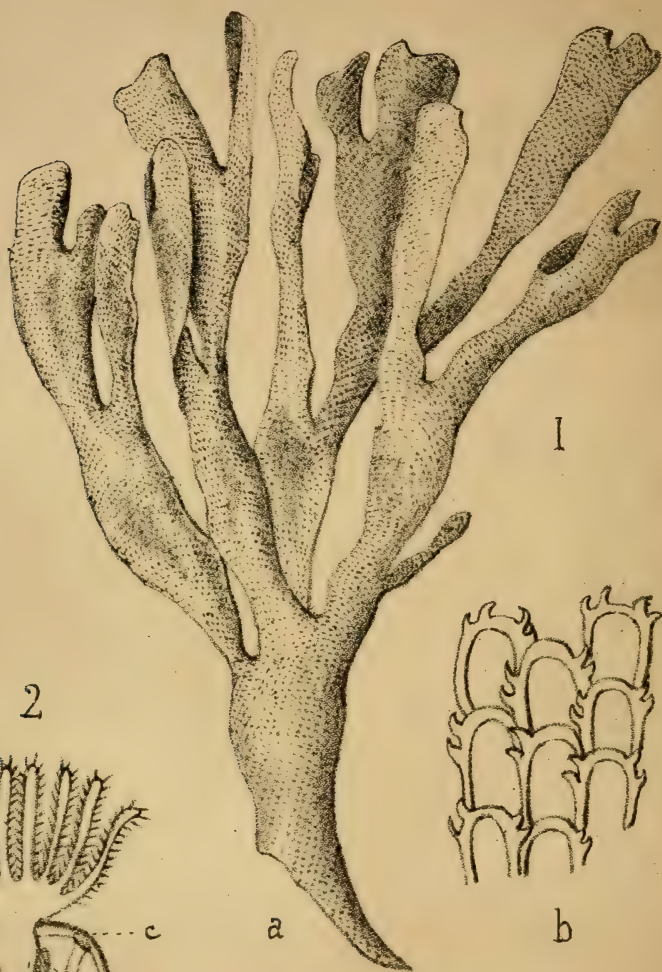
Whatever else the study of the Polyzoa and its long family history may teach, it proves, at least to my satisfaction, that Lamarck's theory of evolution (as I have always understood it) is not supported by what are now well-known facts. We are, however, deeply indebted to Lamarck as the first naturalist who suggested an origin of species apart from a special creative act. An organism of such simple construction as a Polyzoon, with its close method of reproduction, could scarcely be expected to develop into a being very greatly differentiated from the earliest type. It must be borne in mind that in sexual arrangement this organism is hermaphrodite. In the vegetable kingdom, genera which are hermaphrodite, or close fertilising, usually contain a much smaller number of species than those which are mainly or solely propagated by cross-fertilisation.

The *Orchidaceæ*, with a few exceptions, are dependent upon cross-fertilisation, and in some species upon the services of highly-specialised insects, for the performance of this essential operation. Nevertheless, this well-known order of plants is said to contain upwards of 30,000 species. In accounting for the comparatively small variation in structure during such immense periods of geological time, we must remember Darwin's axiom that "there is no evidence of the existence of any law of necessary development." This eminent naturalist* further observes:—

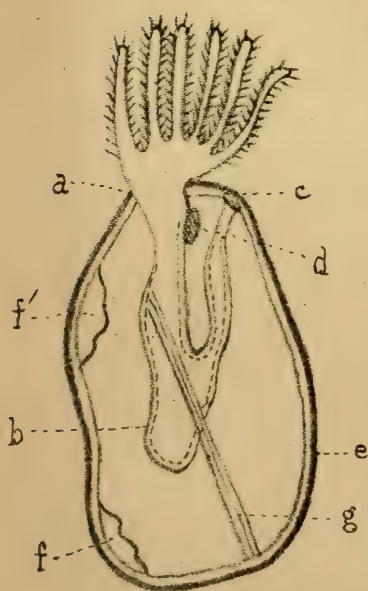
"As the variability of each species is an independent property, and will be taken advantage of by natural selection, only so far as it profits each individual in its complex struggle for life, so the amount of modification in different species will be no uniform quantity. . . . These principles come into play only by bringing organisms into new relations with each other, and in a lesser degree with the surrounding physical conditions. . . . Some forms have retained nearly the same character from an immensely remote geological period, so certain species have migrated over vast spaces, and have not become greatly, if at all, modified."

Bearing in mind the simple structure of the Polyzoa, their reproductive processes, the probability that their very insignificance has in some degree saved them from the severe struggle for

* Darwin's *Origin of Species by means of Natural Selection*, p. 618.



2

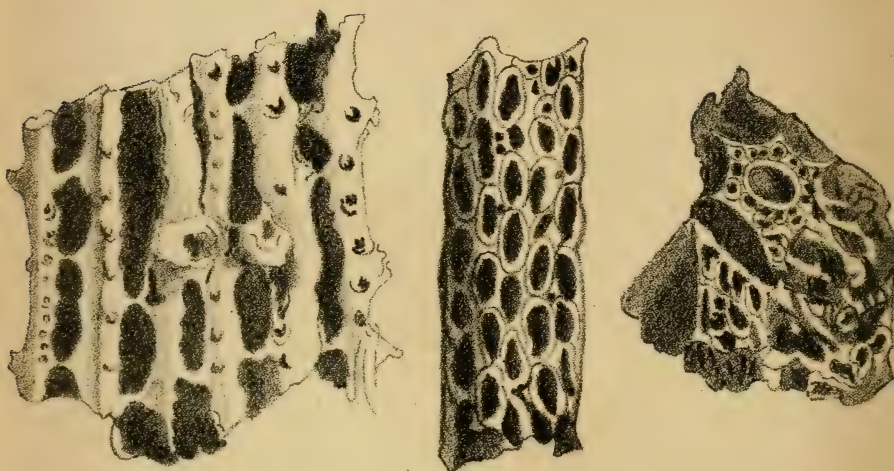


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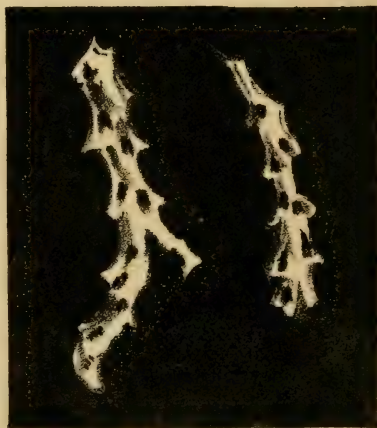




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5



6

existence which so many of their contemporaries have had to endure during the long æons of time which have elapsed since they first became dwellers in the early Primary seas, we need not be surprised to find them at the present day so comparatively little altered in structural character from what they were in those far-off Silurian times. Their history is, in truth, an ancient one. There are few existing forms which can be so accurately traced through these immense geological periods and still be found flourishing in pristine glory in so many of the seas and waters of this globe. The Darwinist may, with good reason, maintain that the small amount of variation and development which this very ancient family has undergone in no way disproves the teaching of his great master, Charles Darwin. The sentences I have quoted from that masterly work, "The Origin of Species," make it plain that Darwin's theory satisfactorily accounts for such seeming anomalies as the history of the Polyzoa presents.

Thoughts like these bring to my mind the words :—

"Flower in the crannied wall,
I pluck you out of the crannies ;
Hold you there, root and all in my hand,
Little flower ; but if I could understand
What you are, root and all, and all in all,
I should know what God and man is."

EXPLANATION OF PLATES XVIII., XIX.

Fig. 1.—*Flustra foliacea*, one of the Sea-mats. *a*, Portion of the colony, natural size ; *b*, A fragment magnified to show the cells in which the separate polypides are contained. After Nicholson.

„ 2.—Diagram of a Polyzoon (after Allman). *a*, Region of the mouth, surrounded by tentacles ; *b*, Alimentary canal ; *c*, Anus ; *d*, Nervous ganglion ; *e*, Investing sac (ectocyst) ; *f*, Testis.

„ 3.—Bird's head process, or "avicularium" of a polyzoon (magnified), after Allman.

„ 4.—Polyzoa, Silurian period. Builders' Beds, Wenloch Shales.

„ 5.—Polyzoa, Carboniferous period, Coal Shales, Scotland (greatly enlarged).

„ 6.—Recent Polyzoa, Bay of Naples.

Drawn by W. G. Wheatcroft.

Clearing and Mounting Sections.*

WHEN sections have been properly stained, and it is desired to preserve selected specimens as permanent preparations, they must be mounted in some medium which will interfere as little as possible with the structure of the tissues and the colours that have been imparted to them. At the same time, by being careful to make use of the most appropriate mounting medium in each particular instance, much aid may be rendered in studying the sections by the increased translucency afforded by this means. Preparations of glycerine or of resinous substances will be found in practice to meet all requirements, and it is often advisable to mount specimens of the same object in both.

GLYCERINE.

Glycerine alone is very awkward to manipulate for permanent preparations, though for temporary examination of vegetable tissues, when somewhat diluted (two fluid parts to one of distilled water), it is a very satisfactory medium. If, however, it be desired to gain the fullest advantage from the use of glycerine it must be used in a more concentrated form, either alone or with as little water as experience shows to be desirable with the particular class of objects in hand at any time. Each section, after being washed in distilled water to remove any alcohol, should be soaked in glycerine. A ring of caoutchouc cement is then made in the middle of a clean slide and allowed to dry. Next, place the section in position within the ring, cover it with a drop of glycerine, give another coating to the cement ring, and having gently breathed upon a clean cover-glass, invert it on the object in such a manner as to avoid introducing air-bubbles. The cover will soon be firmly held by the cement, and any superfluous glycerine may afterwards be washed off the slide by a gentle stream of water from a wash-bottle. Finally, carefully brush round the cover another ring of the cement, and, when this is properly set, the process may be repeated with any finishing varnish that may be desired. If the object is to be mounted in glycerine jelly, as much water as possible should be drained away after placing the section in

* From *The Pharmaceutical Journal and Transactions*.

position on the slide, and the jelly, just sufficient of which has been melted, should be dropped on the section, and a cover, previously breathed upon it as before, placed over it. The slide is afterwards to be set aside until the jelly becomes firm, when the cover may be ringed with Bell's cement. Other convenient preparations of glycerine, which set at the edges of the cover and thus fix it to the slide, contain gum arabic as an ingredient. Hoyer's medium contains in addition, chloral hydrate or acetate of potash, according as it is to be used with sections stained with carmine or hæmatoxylin, or with aniline colours.

CANADA BALSAM.

Of resinous media, Canada balsam is at once the type and the best in use. The raw material is not very suitable, however, since it contains a certain amount of oily matter, which prevents it setting satisfactorily. It is therefore desirable to heat it gently in an oven, until it is of such consistence that it becomes brittle when cold. By then dissolving in benzol, or xylol, in the proportion of about 100 grammes to 50 c.c., it is rendered fit for use. If the menstruum be required to evaporate very slowly, xylol should be employed; but for general purposes the benzol solution will be found preferable. Before these solutions can be applied to the sections, the latter must be dehydrated by means of methylated or absolute alcohol. When the former is employed, the sections must afterwards be "cleared" by immersion in oil of bergamot or oil of cloves, before mounting. After absolute alcohol, however, oil of cedar or xylol will act more satisfactorily. Oil of cloves is very generally used, but it is apt to dissolve out aniline colours and render objects very brittle, if they are left in it very long. As a rule, it is best to leave them in the clearing liquid just long enough to effect the desired purpose (entire removal of alcohol, indicated by the sections appearing perfectly translucent), then remove and mount straightway, by placing upon clean cover-glasses, covering with a drop of the benzol-balsam and immediately inverting upon a clean side which has been slightly warmed to remove the film of surface moisture always present upon glass exposed to ordinary temperatures. If any air-bubbles appear, gentle warming and careful manipulation of the cover-glass with a mounted needle will

generally remove them. Balsam-mounted objects require no ring of cement to retain the covers in position, but the application of one or two coats of Bell's cement will prevent the cedar-wood oil, used with immersion objectives, dissolving out the balsam at the edge of the covers. Further details of processes and full particulars regarding the various solutions, cements, etc., will be found in Lee's 'Microtometist's Vade Mecum,' and Squire's 'Methods and Formulæ.'

Mimicry in Spiders.

MIMICRY is one of the subjects which most secure the attention of the general public in natural history, and one which working naturalists are very apt to notice. No person in any degree, accustomed to take notice of animals and plants, has failed to observe cases of mimicry, whether on the sea-shore, in the forest, or on the prairie: and with the advances of observation we see, in fact, that protective colouration and mimicry are most abundant in nature.

One recent case has been quoted by a French botanist, Professor Heckel, of Marseilles. It concerns a species of spider, *Thomisus onustus*, which is frequently met with in France, where it commonly—at least in the South of France—lives on the common *Convolvulus arvensis*, being very partial to two diptera, *Nomiodes minutissimus* and *Melithreptus origane*, which are frequent visitors to this flower. Professor Heckel has noticed that *Convolvulus* is met under the slightly differently coloured varieties; one is quite white; the second is pink, of a light tint, with some parts of deeper colour; the third is also of a light pink, with some green on the external side. These three varieties are quite common, and live side by side. Now the curious fact is that each of these three forms affords lodgings to three corresponding varieties of *Thomisus onustus*. In the white flowers is found a variety which is white with a little blue cross on the back. In the flowers, which are greenish externally, we find a *Thomisus* which is also greenish, with some pink, and this form lives on the flowers, not in it like the two

other forms. The last variety, pure pink, is inhabited by a *Thomisus*, which is also pink on the dorsal side of the abdomen and limbs. In fact, each of the three colour varieties of *convolutus* is inhabited by a correspondingly coloured variety of *Thomisus*. It has been generally considered that each of the three varieties is a genuine variety; but this assumption is erroneous, as M. Heckel found out. He put in a small box a number of pink *Thomisus*, in order to send them on to a friend for investigation; but he forgot all about the box and its contents during a fortnight, and when he opened it again was astonished at seeing that all the spiders had lost their pink colour. He took some of them and put them on differently coloured flowers, and was much surprised after four days to find each spider had taken the colour of the flower it lived on. As specimens of the same *Thomisus* are often found in the yellow *Antirrhinum* and the red dahlia, he put some of these coloured specimens to these flowers, and saw that they assumed a yellow or red colour. The conclusion is, then, that there are no real and permanent colour varieties of *Thomisus onustus*, but that the same animal may vary in colour according to the colour of the flower it has selected as lodgings.

This fact is very interesting, and we feel inclined to accept it; but M. Heckel has not sufficiently proved his case, as he has not taken care to prevent the possibility of the uncoloured spiders running away and leaving the place to be taken by others. A very simple experiment will easily settle the matter, and perhaps some of our readers may be induced to investigate the subject with other species of animals.

Speaking of spiders, we would call the attention of the farmer to a paper which M. F. Terby has recently published in the *Revue Scientifique*. M. Terby is a Belgian entomologist, and has made some valuable investigations concerning ballooning or flying spiders. Every one has met spiders sailing through the air, and carried on long silk threads. I met some hardly an hour ago, in the bright, warm October day which is closing; and it is in October, when the young spiders are hatched, that the flying spiders are most commonly met with. It would seem that the latter had attracted the attention of Aristotle; at all events, it is quite certain that over two centuries ago Stafford, Martin Lister, and

John Ray described with much accuracy (*Philosophical Trans.*, 1658, 1669, 1690), the manner in which the spiders climb on posts or stalks of grass, crouch down with the abdomen projecting as high as possible in the air, and if some breeze, however slight, is present, send forth jets of silken filaments which float in the air, and are sufficient to carry them off when they let go of the blade of grass or other projecting support. An excellent account of this operation is found in Dr. McCook's admirable *American Spiders and their Spinning Work*, based on the observations of the numerous investigators who have devoted their time to the matter, and on those of the eminent writer himself, but one point has escaped Dr. McCook's attention, in observation as well as in reading; he has not been acquainted with a paper by M. Terby in 1867, and published that year in the *Bulletins de l'Academie Royale de Belgique*, and has not noticed the important fact therein described—that the spider sends forth its jets only under the influence of the motion of the air, and that one may at will induce it to do so by merely blowing on it softly, with the mouth, for instance. As soon as there is some motion of the air the spider, when bent on "moving," of course, seems to be irresistibly impelled to send forth the gossamer. M. Terby's papers will be found useful, as they contain some notes on papers which Dr. McCook does not appear to be acquainted with.

—*Popular Science News.*

In his ascent of Mount Dulit in Borneo, 5,090 feet high, Mr. Charles Hose found a cave above four thousand feet, with wild tobacco growing at its mouth and several remarkable ferns, of one of which the fronds were fourteen feet long. The fauna illustrated the widespread distribution in the islands of Borneo of Himalayan forms. A magnificent view was had from the snow-clad summit of the mountain of distinct ranges. Some natives reported having heard a tiger roaring in the neighbourhood, but Mr. Hose found that the sound proceeded from a gigantic toad, which measured fourteen inches and a-half round the body.

The late Rev. Leonard Blomefield, M.A. (1800—1893).

BY the death, at an advanced age, of the REV. LEONARD BLOMEFIELD, a prominent figure has been removed from the world of science, and another of those links which connect the past with the present has been severed. The deceased gentleman was born in London in 1800 and was the son of the Rev. Leonard Jenyns, a Canon of Ely. He changed his name to that by which he will long be remembered on coming into the Blomefield property in Norfolk. It would appear that he derived his fondness for science generally from his mother, the daughter of the famous physician to the Royal Family, Dr. Heberden. His taste for natural history was fostered by his uncle, Mr. Chappelow. He was first educated at Putney, and went to Eton in 1813, going from there in 1818 to St. John's College, Cambridge. It was at Cambridge where he made the acquaintance of Charles Darwin and Henslow, the botanist. Henslow married his sister in 1823, and was a constant companion in his botanical work. The herbarium which Blomefield commenced about this time gradually grew until in 1887 it consisted of over forty large folio volumes or phanerogams, besides many smaller volumes of mosses, hepaticas, and fresh-water and marine algæ. This collection was deposited in 1869 in the Bath Royal Literary and Scientific Institution.

Having taken his degree he was ordained in 1823 by the Bishop of Exeter and appointed to the curacy of Swaffham Bulbeck, Cambridgeshire, close to his father's Bottisham Hall property. After holding the curacy for five years, he was presented with the living by the Bishop of Ely. Owing to the state of the health of his first wife (Jane, daughter of the Rev. Andrew Daubeny), he left Swaffham Bulbeck, having held the living about thirty years, and, after staying at Ventnor, Isle of Wight, for a short time, he finally settled in Bath. His first wife died in 1860, and in 1862 he married Sarah, daughter of the Rev. Robert Hawthorn, who now survives him. There was no issue by either marriage.

One of Mr. Blomefield's ambitions was to have a good scien-

tific library. While he was at Cambridge his uncle Chappelow died and bequeathed him his library, which contained a large number of books on natural history. His collection of books grew to such unmanageable proportions that in 1869, when he presented his herbarium to the Bath Literary Institution, he determined at the same time to present all the scientific portion of his library to the same institution, conditionally that it should be kept separately from the existing library. He was for many years spoken of as the father of the Linnæan Society (of which he became a Fellow in 1822), in the same year he joined the Cambridge Philosophical Society, he was also an original member of the Zoological, Entomological, and Ray Societies. He joined the British Association for the Advancement of Science in 1832, and the Geological Society in 1835, and was a corresponding and honorary member of many other Societies.

One of the most important of Mr. Blomefield's works is "The Fishes of the Voyage of H.M.S. *Beagle*," published in several parts. In 1836 the Cambridge University brought out his "Manual of British Vertebrate Animals." He published his "Observations in Natural History, etc.," in 1846, and "Observations in Meteorology" in 1858. When his brother-in-law (Henslow) died in 1861, he wrote a "Memoir" of him, which was greatly valued. He was also a frequent contributor of Papers to Scientific Societies and Periodicals, besides writing many valuable criticisms on scientific works.

He founded the Bath Natural History and Antiquarian Field Club in 1855, and was its President at the time of his death. The deceased gentleman will live long in the memories of those who knew him. His courtesy and affability, his readiness to give from his storehouse of knowledge, and his sound judgment in scientific matters, has endeared him to many. Although he was not what is called an advanced scientist, and was cautious in accepting the theories of brilliant but oft-times erratic theorists, yet he will be remembered as a solid worker in science, and the name of Leonard Blomefield will long remain with us, neither will his work be forgotten.

Microscopical Technique.

COMPILED BY W. H. B.

Axis-Cylinder Stains.*—Stroebe's Method.—Stroebe describes a new method of his own for staining Axis-cylinders ; it has proved serviceable in the study of nerve regeneration, and is also quite satisfactory for staining the same structures within the central nervous system. As the fine axis-cylinders of the young nerve fibres, met with in the earliest phases of regeneration, form severe test objects for stains, the present method, in Stroebe's opinion, promises to be of real service. Its special feature is that a practically isolated stain of the axis-cylinder is obtained. Effective contrast staining is possible. The method is as follows :—(1) The tissue is hardened in Müller, thereafter, if desired, in alcohol, and sections are cut as usual. (2) Stain in fresh saturated aqueous solution of anilin blue, ten minutes to one hour ; the sections become blue-black in colour. (3) Wash off excess of stain in water, then place in a porcelain dish of absolute alcohol, to which has been added 20 to 30 drops of 1 per cent. solution of alkali alcohol (1g. caustic potash to 100 c.c. alcohol ; allow to stand twenty-four hours ; filter). In the alkali alcohol sections turn of a rusty-red colour, clouds of reddish colouring matter issuing from them. As soon as these cease to form, and the section is of a light red-brown colour and transparent, differentiation is complete (one to several minutes). (4) Wash in distilled water (five minutes) ; the sections acquire a clear blue tint. (5) Place in the following contrast stain (a quarter to half-an-hour)—concentrated aqueous solution of safranin, diluted with equal parts of water. (6) Place in absolute alcohol to remove excess of safranin and to dehydrate ; the section now looks red with a tinge of blue, use xylol and mount in xylol-balsam. Axis-cylinders appear dark blue ; medullary sheaths, cell protoplasm, ground substance, and cell nuclei, various shades of red. The last named sometimes retain the blue colour.

Van Gieson's Method.—V. Kahlden has a note upon a method of staining axis-cylinders and other structures of the central ner-

* *British Medical Journal*, July 29, 1893, Epitome, p. 20, from the *Centralbl. f. Allgem. Path.*, IV., No. 2.

vous system originally described by Van Gieson. It is as follows: (1) Stain sections—preferably from tissues hardened in Müller's fluid—three to five minutes in hæmatoxylin (Delafield's or ordinary alum hæmatoxylin); wash well. (2) Stain again in a mixture of saturated aqueous solution of picric acid and a saturated aqueous solution of acid fuchsin, sufficient of the latter to make a dark red fluid. (3) Pass rapidly through water, then through spirit, alcohol, and origanum oil; mount in balsam. V. Kahlden has had good results with this method. Axis-cylinders are stained deep red; medullary sheaths, yellow; neuroglia a reddish tint; nuclei, blue or violet; sclerosed tissue, an intense red; hyalin material stains a deep red; colloid, a fainter red or even slightly brown. The relation of amyloid material, which stains a light red to the tissue constituents, especially the vessel walls, is brought out better by this than by any other method.

Preparing and examining Sections of Scales of *Lepidosteus*.*

Mr. W. S. Nickerson, in his interesting paper on the development of the scales of *Lepidosteus*, gives the following method for preparing the sections:—"In all, except very young stages, it was necessary to decalcify the material before it could be sectioned, and, even after decalcification, sections in most cases could not be cut thinner than 15 micromillimetres, and after, in the later stages, it was necessary to make them 20 and 30 micromillimetres thick. For decalcifying, I used 90 per cent. alcohol, to which was added a small quantity of 10 per cent. hydrochloric acid (in the ratio of about 3 to 1). The tissue was usually left in this acid alcohol 24 hours or more, and then soaked in several changes of fresh alcohol to remove all traces of the acid before staining. Sections prepared by grinding down scales have also been studied, as well as scales freed from the soft tissues by treatment with caustic potash. Only by the use of the latter re-agent was I able to get a satisfactory knowledge of the spines which cover the scale in its immature state. The stains which have given the best results are Boehmer's alum hæmatoxylin and Kleinenberg's hæmatoxylin. As a nuclear stain the former is much the better; the latter is especially useful in the study of the glands of the epidermis, and in bringing cell-membranes into pro-

* *Bull. Mus. Comp. Zool.*, Harvard College, xxv. (1893), pp. 117—1193.

minence. . . . The epidermal glands of the second kind . . . are nearly spherical, and have an average diameter of from 18 to 25 micromillimetres. They do not appear to have been recognised by previous observers. Some of these lie in the deeper part of the epidermis, but much the greater number and the larger ones occur near the surface, where many of them are open. They stain deeply with Kleinenberg's and with Delafield's hæmatoxylin, but in all other stains used, even in other hæmatoxylin dyes, the nuclei alone are coloured, and in this condition they can be distinguished from small glands of the first kind only by very careful observation. This probably accounts for their having been overlooked heretofore. In Kleinenberg's hæmatoxylin the whole gland takes a blue tint, while the wall presents a reticulated appearance due to an irregular network of lines of a much deeper blue colour. . . . Hæmatoxylin has long been known to stain mucus deeply. Hoyer ('90) found that basic stains are those which chiefly affect mucin, and Delafield's (alum) gave characteristic deep blue stains to these glands; Boehmer's (alum) and Ehrlich's (acid), on the other hand, gave pure nuclear stains. Hoyer imputed certain failures of hæmatoxylin stains to act in their normal manner to lack of ripeness, and it is possible that this may be the reason for Boehmer's alum hæmatoxylin not colouring the mucin in this case."

Preserving and Examining Copepoda.*—As a preservative fluid for Copepoda, Mr. J. C. Thompson finds that a mixture composed of equal parts of alcohol, water, and glycerine, with 1 per cent. of carbolic acid, is most useful. Specimens may be transferred direct to it from sea water, and can be so preserved for any desired period of time, to be mounted direct, without further preparation, in either glycerine jelly or Farrant's medium.

Mr. Thompson employs an exhaustive method for capturing these organisms with the greatest advantage, particularly when material, or dredged mud or sand, is kept a considerable time before it is examined. The dredged material is washed through a coarse sieve into a finely meshed silk bag, into which a running stream of water from a tap is allowed to fall. By careful kneading

* Revised Report on the Copepoda of Liverpool Bay, by J. C. Thompson, pp. 4, 5 (*Trans. Liverpool Biol. Soc.*, Vol. VII., 1893).

with the hands, all the soluble or very finely suspended particles are washed away through the texture of the bag. The clean residue is then placed in a large flat dish of water and stirred round, when the fine floating organic portion, often very rich in Foraminifera, Diatomacea, Ostracoda, Amphipoda, etc., can be strained off and placed in a preservative fluid for examination.

Method of Preserving Rotatoria.*—Mr. C. F. Rousselet having made many experiments in order to preserve the rotifers, says the following method has proved very successful in his hands. The process consists of four stages, viz., narcotising, killing, fixing, and preserving.

NARCOTISING.—A fluid eminently suitable for this purpose is a watery solution of 1 to 2 per cent. of hydrochlorate of cocaïn. A small quantity of this solution, added to the water in which the rotifers are, does not at first affect them, but, after some minutes (5 to 15), their motion becomes slower, and, in successful cases, they finally sink to the bottom of the trough fully extended, with the cilia vibrating but feebly. It is necessary to watch them until the cilia have just ceased to vibrate, and then, in the majority of species, is the right moment to kill. The action of cocaïn varies greatly in different rotifers, so that experience is necessary to be able to judge as to the right quantity required.

KILLING AND FIXING.—Both these operations are performed simultaneously by adding a small quantity of Flemming's chromo-aceto-osmic acid mixture (15 parts of 1 per cent. chromic acid, 4 parts of 2 per cent. osmic acid, 1 part of glacial acetic acid). The animals remain in the fixing solution a quarter to half an hour, not longer; small rotifers rather less. The solution is then washed out with distilled water by changing the water five or six times.

PRESERVING.—Mr. Rousselet finds that the best preserving fluid is simply distilled water rendered antiseptic by a trace of the fixing solution (8 drops to 1 oz. of water).

Nature of the Staining Process of Vegetable Tissues.†—It has long been a matter of controversy whether the colouring of

* *Journ. Quekett Micr. Club*, II., v. (1893), pp. 205—209.

† Dingler's *Polytechn. Journ.*, 1893, Heft 9.

cellulose fibres by staining re-agents is dependent on a chemical process, or merely on a mechanical union of the particles of the pigment with those of the cellulose. Herr G. Spohn has attempted to set this matter at rest by a careful microscopical examination of cotton-wool stained by mineral pigments. He found no change whatever in the structure of the fibre itself. Even when the fibres were macerated before staining with alizarin, they acted sharply as a carrier of the pigment, on which the macerating fluid acts chemically. In all cases, according to these observations, the combination of the pigment with the fibre depends entirely on purely mechanical causes.—*Pharmaceutical Journal*.

Mounting Spores of Equisetum.*—Mr. T. D. Schoonmaker gives his method for mounting these spores as follows:—Take a zylonite or rubber cell, $\frac{3}{4}$ of an inch in diameter and about $\frac{1}{8}$ of an inch, or a little less, in depth, and glue it fast to the slip. After it has become firmly attached to the glass, cut with a file several creases in the cell, half the depth of the cell, or about $\frac{1}{16}$ th of an inch. Spread the spores evenly in the cell, and attach the cover-glass by spreading on a little marine glue between the filings. This method confines the spores and allows enough moisture from the breath to pass through the triangular openings to set the spores “dancing.”

Mounting Medium for Algæ and Fungi.†—Dr. A. A. Julien recommends the following solution, an indirect outcome of Ripart and Petit’s formula, for mounting organisms with endoplasm of ordinary density, e.g., most of the filamentous algæ:—Copper chloride, 0.1 gm.; copper nitrate, 0.1 gm.; chloral hydrate, 0.5 gm.; distilled water, just boiled, 100 ccm. The trace of acidity is removed from the solution in the following manner:—Another solution is prepared of a few grms. of any soluble copper salt; to this a weak solution of caustic potash is added in slight excess; the hydrated copper oxide is then washed thoroughly, first by decantation, and then upon a filter. The purified residue is then thrown into 100 ccm. of the preservative fluid already prepared,

* *The Observer*, IV. (1893), pp. 198—199.

† *Journ. R. Micr. Soc.*, 1893, p. 566, from *Journ. New York Micr. Soc.*, X. (1893), p. 39.

and the mixture frequently shaken at intervals until a neutral reaction is shown by test papers when it is filtered.

Simple Method of Plate-Culture.—Dr. R. Boyce, of University College, describes in *Public Health* a simple method employed by him for obtaining thin plate cultures. It consists in placing an ordinary glass slide in a wide test-tube, at the bottom of which is placed some moist cotton wool. After being also plugged with cotton wool, the tube with its contents is sterilised, by being exposed for twenty minutes to a temperature of 120° C. in an autoclave, or for three-quarters of an hour in ordinary steam. The sterile nutrient medium is kept ready for use in a siphon pipette, and can readily be spread upon the slide, after being melted by warming, without risk of contamination, if the tube be held horizontally whilst the plug is being removed and the slide coated. The plug should then be rapidly passed through a flame and replaced in the tube, which ought to remain in the horizontal position until the thin film of nutrient medium on the slide is set. If a rubber cap, rendered aseptic by immersion in corrosive sublimate solution, be then drawn over the mouth of the tube, the prepared slide may be kept ready for use for an indefinite period. Inoculation is performed in the usual way, and incubation effected in the tube. Afterwards the slide can be readily examined under the microscope, whilst the specimen can easily be fixed in alcohol and stained with gentian violet if necessary. On account of the simplicity, economy of material, and convenience associated with this method, it appears to possess advantages over the older systems.

Estimation of Fat in Milk, by Weiss.*—Thirty c.c. of milk is introduced into a flask of 300 c.c. capacity, and then mixed with 3 grams of officinal sodium hydroxide solution; light petroleum (60 grams) is added in three portions, and the mixture shaken after each addition until the liquid is quite homogeneous. If the last portion of petroleum does not mix readily, the liquid is placed aside for a quarter of an hour, after which mixing is easily brought about. The emulsion may be kept without separating for a day, but after adding 20 grams of alcohol, and shaking the liquid frequently, sepa-

* *Journ. Chem. Soc.*, No. 369, 1893, p. 396, Abstracts.

ration is effected in six hours, and, at the end of twenty-four hours, three liquid layers have formed. The lowest is clear, and of a yellow colour, the middle one somewhat turbid, whilst the upper one, which is clear and colourless, contains the whole of the fat. An aliquot weight of the latter (petroleum solution) is then evaporated in a flat dish at 50° , and the residue dried at 100° . Instead of weighing the fat solution, 25 c.c. of the milk is shaken with 3 c.c. of sodium hydroxide solution, and subsequently with three portions of light petroleum (25 c.c. each). 50 c.c. of the fat solution is then evaporated, and the fat weighed and calculated to 100 c.c. of milk.

The Brownian Movement.*—Mr. A. B. Chapin gives the following method for making a slide showing the Brownian Movement:—Take two perfectly clean one-ounce bottles; into No. 1 put half an ounce of distilled water, ten drops of the tincture tolu, four drops of pure carbolic acid, and filter through absorbent cotton into No. 2. Make a very thin glass cell, and cement it to the slide with hard marine glue. Now put the slide on turn-table, and spin a very shallow ring of *good* gold size on the outer edge of the cell, and allow it to dry until just ‘tacky’ enough to adhere well to the cover-glass. Put into this cell enough of the solution to fill, carefully adjust the cover-glass, and clamp down very lightly. Absorb any surplus that may have run out with blotting-paper, set aside for a few days, then ring, and you have a permanent mount.

Air-Bubbles and Oil-Globules.†—It is of importance to be able to identify and distinguish between air-bubbles and oil-globules in preparations under microscopical investigation. The appearances of both vary considerably according to the portion of them that happens to be in focus. Dallinger in *The Microscope and its Revelations* represents and describes these different aspects, as presented when light is transmitted from a concave mirror exactly centred (axial illumination), and a diaphragm of about two-thirds of a mm. is placed at a distance of 5 mm. beneath the stage. This will represent the smallest opening if a wheel diaphragm be used, whilst an iris diaphragm should be almost completely closed. Air-bubbles in water and Canada balsam respectively may be

* *The Observer*, IV. (1893), p. 199.

† *Pharmaceutical Journal*.

examined in a drop or two of either liquid, placed upon a slide with a thin cover superposed after vigorously shaking the bottle containing it. A drop of oil of turpentine coloured with magenta or carmine, and a drop of water, may be placed on a slide together, covered, and the cover moved about to cause them to mingle. Globules of oil in water may also be studied in an emulsion prepared by shaking the two together with a little powdered gum. In an air-bubble in water, when the middle of it is focussed, the centre of the image appears very bright, and it is surrounded by a greyish zone, which in turn is encircled by a broad black ring interrupted by one or more brighter ones. Outside the black ring are diffraction circles, brighter than the field. On focussing downwards, the bright centre becomes smaller and brighter, and is sharply divided from a very broad black ring which has bright diffraction circles outside. Upward focussing, on the other hand, causes the central portion to increase in size but become less bright, whilst the now narrowed black ring is surrounded by numerous diffraction circles. Air-bubbles in Canada balsam have similar appearances in the different positions to those in water, but, on account of the high refractive index of the balsam, the bright central circle is smaller in each case. An oil-globule in water shows the central disc brightest when the upper part of it is in focus, and the broad black outer circle is not surrounded with diffraction rings. Focussing down to the middle of the globule, the disc becomes very large, but is much less bright, and the narrow black encircling ring is bordered by diffraction circles both within and without. On lowering the objective yet further the bottom of the globule appears as a grey disc, somewhat darker than the field, and separated from it by a darker ring.

Handsome Cell-material.—Celluloid.—A writer in the *National Druggist* (XXIII., 1883, pp. 28 and 42) gives the following description of a method for using celluloid as a cell material:—"Having at hand a few sheets of celluloid imitation ivory of high grade, the idea struck him that this was the very thing, and a little experimentation proved that the idea was correct. The sheets were not thick enough to make a cell of the required depth, but this was no drawback, since celluloid is easily cemented. With sharp punches of proper size he cut out a sufficient number of rings from the sheets

of celluloid. This is easily done if the material be laid on a thick and smooth block of lead, and the punches be kept sharp. Moistening the surfaces of the rings with a mixture of alcohol and ether (or absolute alcohol will answer) until a ring of sufficient thickness was built up, the composite ring was placed under a weight and allowed to dry. In the cells intended for specimens for direct examination, the bottom was formed of a disc of celluloid of the same external diameter as the rings, and the rings were then cemented to it. The internal diameter of the upper ring of the series was made about one-tenth of an inch larger than its fellows, in order to form a seat for the cover-glass. The roughness was removed from the external portion of the cell by placing the slip to which it was cemented on the turn-table, and revolving it first against a file and afterward against a bit of crocus-cloth, cemented to a smooth piece of wood. Finally, a high polish was given by rotating the ring against a little silk pad, wet with absolute alcohol, and then rubbing it with silk greased with olive oil. When finished, the cell resembled one turned from solid ivory. After the cells were filled with material, they were closed by dropping the cover-glass into the recess prepared for it, the edges of which were touched with absolute alcohol before dropping the cover-glass into place. The operation was finished by cementing a final ring of celluloid so as to cover the joint made with the glass. The cells thus made, while apparently tedious to prepare, are very handsome, and do not actually take much time to construct. It will not do to use alcohol and ether mixture to fasten the cells to the slip. The joint, while perfect at first, soon separates, and the cells drop off the glass. White zinc cement, diamond cement, or marine glue, makes a firm joint. The alcohol and ether mixture, however, makes a firm joint of celluloid to celluloid. . . . For the reason given, it is best to fasten the cover-glass to its seat (if an air-tight joint is desired) with gelatin or any of the cements mentioned."

Balsam-paraffin for Cells.*—Dr. A. A. Julien observes that the mixture of balsam and paraffin for making cells deserves to be better known. Balsam cement is first prepared by slow evapora-

* *Journ. R. Micr. Soc.* (1893), p. 567, from *Journ. New York Micr. Soc.*, ix. (1893), pp. 39—43.

tion of commercial Canada balsam in a shallow tin pan over a low flame, until the point is reached of wax-like consistence on cooling, as tested on drops removed and tested from time to time. About a quarter of a pound of the hardest commercial paraffin, melting point above 45° C., is heated over a low flame to the melting point, a piece of balsam-cement (size of a nut) is then added, and the mass digested with frequent stirring for about an hour, until all the paraffin has a slight yellowish tinge. The stock is preserved in a shallow porcelain capsule, so that when required it can be readily warmed up. A cell made with this paraffin-balsam is ready for use directly after it is spun.

Mounting Sections in Canada Balsam.—The most satisfactory method of mounting specimens permanently in Canada balsam is undoubtedly that originated by Cole some years ago. It is cleanly, the various operations may be performed deliberately, and there is practically no risk of spoiling a preparation during the process of mounting. The process as described in Cross and Cole's *Modern Microscopy*, just published, has been somewhat modified since originally published, and is now simplified and improved. After the section has been properly cut and stained, it is directed to be cleared by floating in oil of cloves for about five minutes, and then transferred to turpentine. The Canada balsam should have been previously prepared by dissolving three ounces of the dried balsam in three fluid ounces of pure benzol and filtering. A glass slide having been breathed upon, a clean cover-glass is applied to the moistened surface so as to adhere to it. A little benzole-balsam is then placed on the exposed side of the cover, and the section immersed in it. The difference in the result may be very slight or even nothing, but it is just possible that by first spreading the section on the cover and then covering it with the medium, it may be left closer to the cover in the finished slide, and so be more advantageously placed for examination with high-angled objectives, which have correspondingly short working distances. The risk of air-bubbles in either case is extremely slight. The slide is afterwards covered with a bell-glass, or otherwise protected from dust, and left for twelve hours for the benzol to evaporate. A drop of fresh benzol-balsam is then added to that enclosing the section,

cover inverted by means of a pair of forceps, and lowered on to the middle of a clean slide that has previously been gently warmed in a spirit-lamp flame. If the cover be lowered carefully and gradually, any air-bubbles that may have formed will escape easily, and the cover may then be pressed steadily until the section lies quite flat and uniform. As soon as the slide is quite cool, any exuded balsam may be washed off with a soft rag or camel-hair brush moistened with methylated spirit, and, after a final polish with a dry cloth, a ring of cement may be applied to finish. Thus, though some time is required for the balsam to harden in the preliminary stage of this process, that is much more than compensated for by the rapidity with which the slides can be finished off. In the ordinary, so-called quick method, the slides must be left for a day or two, or even longer, before it is safe to attempt to clean and ring them, and there is always a risk of moving the cover or admitting air-bubbles at the last moment.

Clearing Sections.—The object of this process, as is well-known, is to remove all traces of alcohol from dehydrated sections. Unless this be completely effected, the remaining alcohol will cause a certain amount of cloudiness where it comes in contact with the Canada balsam subsequently used as the mounting medium. The essential oils of bergamot, cedar, and cloves, are generally used for the purpose of clearing, the last-named most commonly perhaps. Bousfield, however, in his *Photo-Micrography*, notes that he has long ceased to use essential oils after dehydration. He points out that however high the refractive index of the oil used may be, the ultimate index must be that of the medium used for mounting. In his own particular case this is xylol-balsam, and he prefers to remove the last traces of absolute alcohol from the sections by immersing them in two successive lots of mineral naphtha. This he finds to answer every purpose, and the fact that the fluid is entirely without action upon aniline dyes, with which staining is now so often effected, gives it a special advantage over most essential oils, particularly that of cloves.

Crystals of Gold.—To procure gold-crystals, as objects for the microscope, proceed as follows:—Make a 10 per cent. aqueous solution of neutral auric chloride (Au. Cl. 3), and of this put a

drop on a glass slip, spreading it somewhat with a glass rod. Touch the drop with a piece of zinc plate cut to a point, pushing the point well towards the centre. The crystals will form in feathery masses, which make a very beautiful object for the microscope.

New Multiple Staining Fluid.—The Sheffield *Medical Journal* says that Unna differentiates bacilli in tissues by a polychromic methylene blue solution, which contains methylene red and violet in addition to the blue. The sections are transferred from alcohol and allowed to remain in the stain for at least ten minutes. They are then passed through water into 33 per cent. tannic acid solution to decolorise, allowed to remain from two to five minutes, then rinsed with water to enable the exact tint to be observed more readily. If satisfactory, after a thorough washing with water, the sections are placed in absolute alcohol, or a solution of gold orange in the same if a yellow counter stain be desired, cleared in oil of bergamot, and mounted in balsam.

If the excess of stain is not readily removed, a few minutes' immersion in 25 per cent. nitric acid, followed by dilute spirit, water, and absolute alcohol respectively, will effect its removal. By adopting this method, it is said to be possible to distinguish two kinds of nuclei (violet and blue)—the fibrine and the protoplasm of the plasma-cells. The bacilli stain red, whilst the mucus surrounding them is blue, and the organisms are said to appear in their natural character "in fish-roe-like masses of vegetable mucus." It is claimed that the process is particularly suitable for use in the study of leprosy." It appears to depend upon the property, also utilised by Nicolle, by which tannin converts methylene blue into an insoluble form.

A LIVING ILLUSTRATION of the truth of the evolution theory has been dredged in 392 fathoms off one of the Galapagos Islands, in the shape of a stalked crinoid, or sea lily, in which are united the characteristics of three distinct fossil genera of the same group of organisms:—*Apiocrinus* of the Bradford clay deposits, *Hyo-crinus*, and *Rhizocrinus*. This interesting survival of a very old and complex type will shortly be described by Mr. Alexander Agassiz.—*The American Naturalist*.

Notes.

THE POSTAL MICROSCOPICAL SOCIETY.—The twentieth Annual Meeting of the Society will be held at Cambridge on Saturday, October 7th, and it is hoped that as many members as can make it convenient will be present.

Members are invited to take Luncheon with G. H. Bryan, Esq., the retiring President, at St. Peter's College, at two o'clock. After Luncheon, J. W. Fisher, Esq., of Lyme Regis (late of Ealing), will read his Presidential Address. Then a short business meeting will be held to receive the Hon. Secretary's Report and for the election of President for the session 1894—5.

From 3.30 to 5.30 it is proposed that the party will visit the principal Colleges and other places of interest in Cambridge, and at 5.30 they are invited to afternoon tea at Mr. Bryan's rooms in St. Peter's College.

We believe the visit will be a very enjoyable one for those who can attend.

Mr. C. F. Rousselet has lately published (*Journ. Roy. Micro. Soc.*, 1893, pp. 450—458) a most valuable list of all the new rotifers that have been found since the last supplement to Hudson and Gosse's *Rotifera* in 1889. As Mr. Rousselet truly remarks, there is no doubt that the publication of that splendid monograph has given an immensely stimulating effect to workers on these beautiful organisms. He enumerates 186 new species, but thinks it probable that some of these have been named twice over. In looking through the bibliography which he appends, it would appear that there are few observers of rotifers to be found in France, Italy, and Spain. It seems also that our friends in America do not pay much attention to them, as we only notice two observers in the list. Perhaps this is an error on our part, but we should have imagined that there are many undescribed forms in the ponds and ditches of those countries.

Our contemporary, *Nature* (XLVIII., p. 297), has the following note *apropos* of the Owen Memorial Fund :—

“When it was resolved last January ‘That it is desirable that the eminent services of the late Sir Richard Owen in the advancement of the knowledge of the sciences of anatomy, zoology, and palæontology should be commemorated by some suitable memorial,’ it was confidently expected that there would be a generous response to the appeal for funds. A large number of circulars were sent out; yet the list published in June contains the names of

less than 300 contributors. The donations then amounted to £935, and the amount promised has even now only reached £1,000, whereas the committee hoped to obtain at least twice that sum. For those who have come forward there is nothing but praise; the cause of complaint lies in the paucity of subscribers. Only 300 admirers of Owen can be found desirous of giving concrete expression to their feelings of regard. The fact is humiliating, and, for the sake of British science, we trust it will soon be altered. Of Sir Richard Owen it can truly be said that among students of science 'many shall commend his understanding, and as long as the world endureth it shall not be blotted out; his memorial shall not depart away, and his name shall live from generation to generation.' But Owen's greatness should not only be appreciated by men of science; it should be made known to the world by means of a monument. As a mark of respect to their master and an act of duty, all naturalists should add a stone to his cairn."

We trust that since the above was written (in July) that the fund has considerably increased. When we first heard that a fund had been started, we hoped that there would have been such an amount of money forthcoming as would have enabled the committee to have founded an Owen Scholarship in Comparative Anatomy as well as the intended statue at South Kensington.

Although Mr. C. Bendire, in his valuable and suggestive paper, writes only for American oölogists, his remarks are equally applicable to collectors elsewhere. He says:—

"Unless the would-be collector intends to make an especial study of oölogy, and has a higher aim than the mere desire to take and accumulate as large a number of specimens as possible, regardless of their proper identification, he had better not begin at all, but leave the nests and eggs of our birds alone and undisturbed. They already have too many enemies to contend with, without adding the average egg-collector to the number. The mere accumulation of specimens is the least important object of the true oölogist. His principal aim should be to make careful observations on the habits, call-notes, song, the character of the food, mode and length of incubation, and the actions of the species generally from the beginning of the mating season to the time the young are able to leave the nest. This period comprises the most interesting and instructive part of the life-history of our birds.

Do not start with the idea that because a certain species may be common with you everything must consequently already be known about it, and that your observations would be useless.

Rest assured that some new and interesting fact can still be learned by the observant oölogist about even our commonest birds.

A small, thoroughly identified, well prepared, and neatly-cared-for collection, even if only a local one, is worth far more scientifically, and in every way, than a more extensive one obtained by exchange or purchase."

TO COLOUR ARTICLES OF CELLULOID.*—According to the *Gummi Zeitung*, celluloid can be stained almost exactly like ivory. The following are some of the formulæ suggested:—

Black.—Plunge the article first in weak lye and then into a weak solution of silver nitrate, and let dry in a strong light. *Blue.*—A solution of indigo, rendered nearly neutral by the addition of potash, gives indigo blue. Prussian-blue solution, or, better still, a bath of iron chloride, followed by one of ferrocyanide of potassium, gives a dark blue. *Green.*—A solution of verdigris. *Yellow.*—Dip first in a solution of lead nitrate, and then into a solution of yellow potassium chromate. *Brown.*—Solution of potassium permanganate rendered alkaline with soda. *Red.*—Dip first in a weak solution of nitric acid and then into an ammoniacal solution of carmine. *Purple.*—Dip into a solution of gold chloride and place in the sunlight.

AN EXCELLENT LABEL PASTE FOR SLIDES.—Having experimented a good deal with the view of getting a paste that will not separate from the slide, the *National Druggist* (XXIII., p. 6) says, we can heartily recommend the following:—

Dissolve four ozs. of picked gum arabic in eight ozs. of water. In another vessel make a paste with 1 oz. of gum tragacanth in 4 ozs. of water. Mix the solution and the paste and strain the mixture through a linen handkerchief. To the colate add 2 ozs. of glycerine, in which has been dissolved 30 grs. of corrosive sublimate, and mix well. Thus prepared, the paste will keep almost indefinitely, and it will stick paper to almost anything. Some slides labelled with it several years ago still retain their labels firmly attached, while the labels put on with gum arabic, dextrine, etc., have all loosened.

JERSEY BIOLOGICAL STATION.--This station, which comprises Laboratory, Aquarium, Scientific Reference Library, and Type Museum, is now completed and ready for the reception of students

* *National Druggist*, XXIII. (1893), p. 27.

and of those wishing to prosecute research work. The Station is built on the sea front, at a level of 18ft. above tide-mark, and it overlooks one of the largest and most prolific grounds for shore-collecting in these latitudes. At low-water an area of not less than twelve square miles of rock-pools and *Zostera* prairies (constituting "*Le Banc des Violets*") is exposed and made accessible by foot, while the still almost zoologically-unexplored Minquier Reefs (nine miles south of the Station) are easy of access. Every facility is given for trawling, dredging, etc. Two boatmen, well experienced in naturalists' work, are in attendance, and a small steamer is also available at a day's notice. Applications for terms should be addressed to Messrs. Sinel and Hornell, Jersey Biological Station, Jersey (Channel Islands).

VILLARSIA NYMPHOIDES.—Will any reader inform me in what locality this member of the Gentian family is found? I wish to obtain a piece of the stem for cutting sections. I believe it is a river plant. Who will kindly send me a small piece?—J. Phillips, 16 Alexandra Crescent, Leeds.

Reviews.

FLORA OF SOUTH-WEST SURREY. By S. T. Dunn, B.A. Cr. 8vo, pp. xv.—106. (London: West, Newman, and Co. 1893.) Price 3s.

This admirable little flora will supply the information which the botanical visitor to Leatherhead, Dorking, Guildford, Godalming, Farnham, and Haslemere must hitherto have sorely needed. It is up to date, and combines all the essentials of a good local Flora, with portability of form. We congratulate Mr. Dunn on the success with which he has accomplished the task he set himself.

AN INTRODUCTION TO THE STUDY OF GEOLOGY. By Edward Aveling, D.Sc.Lond. Crown 8vo, pp. viii.—354. (London: Swan Sonnenschein and Co. 1893.) Price 6s.

A most useful work, specially adapted for the use of candidates for the London B.Sc. and the Science and Art Department Examinations, the syllabus of this department being taken as a basis for the general plan of the work. It contains a coloured map, 132 good illustrations, and a glossary. The student will find this book most helpful.

THE ESOTERIC BEAUTY and Utility of the Microscope. By Ephraim Cutter, A.M., LL.D. 8vo, pp. 60. (New York: 120 Broadway.) Price 40c.

The author gives as a principal reason for writing this pamphlet his having heard a certain divine repeatedly use the expression "accursed microscope." He confines his remarks as to its utility chiefly to its valuable aid in medicine and surgery.

INTRODUCTION TO THE STUDY OF THE DIATOMACEÆ. By F. W. Mills, F.R.M.S.; with a Bibliography by Julien Deby, F.R.M.S. 8vo, pp. xi.—243. (London: Iliffe and Son. 1893.) Price 12s.

The author describes the Structure and Movement of Diatoms; their Classification, with a conspectus of the families and genera; modes of reproduction; collecting and mounting diatoms; the microscopical examination of diatoms; how to photograph diatoms; and a bibliography relating to diatomology.

The work is handsomely got up, but we regret to notice a great number of typographical errors, which we trust will be corrected in a second edition.

A DICTIONARY OF BIRDS. By Alfred Newton, assisted by Hans Gadow. Part I. 8vo, pp. viii.—304. (London: A. and C. Black. 1893.) Price 7s. 6d. net.

Although the work before us does not profess to be a complete treatise on Ornithology, it will doubtless be welcomed by the ornithologist. The author takes as his groundwork the series of articles contributed to the last edition of the *Cyclopædia Britannica*, which he works up according to an alphabetical arrangement. The work is to be completed in four parts. We hope to refer to it again—most probably in January.

THE BIRDS OF LONDON. By H. K. Swann. Fscap. 8vo, pp. 136. (London: S. Sonnenschein and Co. 1893.) Price 2s.

A short description is given of all the species of birds which have occurred more or less frequently within a radius of twelve miles of London. These are described by their scientific as well as English names; we are surprised to find they number upwards of 230.

ILLUSTRATED BIBLE DICTIONARY. By M. G. Easton, M.A., D.D. Crown 8vo, pp. xi.—724. (London: T. Nelson and Sons. 1893.) Price 5s.

We have here a treasury of Biblical history, biography, geography, doctrine, and literature. It is a complete and trustworthy book of reference on all Biblical subjects, and will be found invaluable to the Bible student. It contains 200 illustrations, besides maps and plans.

THE UNRIVALLED ATLAS of Modern Geography. (Edinburgh and London: W. and A. K. Johnston. 1893.) Price 5s.

Contains forty well engraved and coloured maps, size 14½ by 12 inches, and an index to 20,000 names contained in the maps. There are also letter-press explanations of the Classical and Physical Maps and of the Solar System and Seasons.

SCHOOL GEOGRAPHY AND ATLAS. By G. Carter, M.A. 4to. (London: Relfe Bros.).

A great deal of useful information is compressed into this book, which contains 32 maps, the maps being on one page and the geographical notes on the opposite. The maps are drawn in such a manner that they may be readily reproduced by the scholars, and they are *not* overcrowded with names.

THE GREAT NORTH ROAD MAP. Compiled by H. R. G. Inglis. (London: Gall and Inglis.)

As this map folds into 6 in. by 3 in., it will be found very convenient for the traveller. The distances of all the principal towns from either end of the route are plainly marked. The names of towns and villages are engraved distinctly.

DELLA LEPTOTHRIX RACEMOSA : Terza memoria sulla Flora Crittogamica Della Bocca. By F. Vicentini, M.D. (Napoli : A. Tocco and Co. 1893.)

The volume before us is the continuation and development of two preceding memoirs on the same subject.

In the first part the author gave some recent bibliographical notices on the micro-organisms of the mouth, and an exposition of the analogies of the inferior phases (of which the forms only have been hitherto known) of *Leptothrix buccalis*, with other bacteria (*Cladothrix dichotoma*, *Bacterium Balbiani*, *Bacterium osteophilum*, and *Leptothrix parasitica*, Kützing.)

In the second part the author proposes to change the name of *Leptothrix buccalis* into that of *L. racemosa*, in order to designate its fructification or sporulation, which he has detected on the outside of certain filaments. He subsequently describes his new and original observations made on the various elements of the fructification, viz. :—1.—The fertile filament or central stem, with reserve gemmules inside. 2.—The peduncles or sterigmata. 3.—The sporules. 4.—The surrounding glia, or gelatinous protective envelope, which appears to warrant the delicate texture of the fruits.

The author states that the conical peduncles, *sterigmata*, or threads, by which the sporules are implanted on the outside of the central stem, could not be observed by a lower power than $\frac{1}{25}$ th homogeneous immersion. He describes also other special forms of the filaments and fructification, and of certain structures, which he believes to be male organs (antherids or spermogones), of this complex micro-organism, which bear analogy both to the algæ and to the fungi.

The common bacteria of the mouth must, therefore, according to the author, be referred to inferior phases of the life of the same micro-organism—viz., the filiform, the dissociated, or the zoogleic state, or to its sporules detached from the female filaments; or they must be referred to fecundating elements (antherozoids or spermata), detached from the adult male organs, and quickly swimming in the vehicle.

The Monograph is accompanied by a fine coloured plate, consisting of 14 figures.

RECENT DEVELOPMENTS IN MASSAGE. By Douglass Graham, M.D. pp. iv.—128.

ELECTRO THERAPEUTICS AND NEURASTHENIA. By W. F. Robinson, M.D. pp. x.—72.

THE BACTERIAL POISONS. By Dr. N. Gamaleia. pp. xiii.—136. Foolsap 4to. (Detroit, Mich. : Geo. S. Davis. 1893.) Price 1s. each, paper covers; 2s., cloth.

The above vols. of the "Physician's Leisure Hour" series will doubtless prove of much interest to the profession. The volume on Massage is well illustrated, but that on Bacterial Poisons will probably prove of greater interest to the general reader. The first part treats of Experimental Study of the Putrid Poisons, The Discovery of Ptomaines, etc.; Part II. of The Chemical Nature of the Bacterial Poisons, The Origin of the Microbian Poisons, and Action of the Bacterial Poisons on the Animal Organisms, etc.; Part III. of the Poisons of Tetanus, Diphtheria, Cholera, etc.

THE RECRUDESCENCE OF LEPROSY and its Causation. By William Tebb. Crown 8vo, pp. 412. (London : S. Sonnenschein and Co. 1892.)

Since 1884 the author has made a careful study of this fearful disease and of the causation of its increase, which he attributes in a great measure to vaccination. In an appendix he denounces vaccination in very strong terms.

MY WATER-CURE. By Sebastian Kneipp. Second edition. Crown 8vo, pp. xxxiii.—282. (Edinburgh and London: W. Blackwood and Sons. 1893.) Price 3s. 6d.

The volume before us is the translation of a German work by Sebastian Kneipp, parish priest of Worishöfen in Bavaria, in which he describes his celebrated water-cure, which he has now practised for many years with great success. The work contains much that is worth reading.

REACTIONS. By F. A. Flückiger, Ph.D., M.D.; translated, revised, and enlarged by J. B. Nagelvoort. Royal 8vo, pp. x.—154. (Detroit, Mich., U.S.A.: Geo. S. Davis. 1893.) Price \$2.

Contains a selection of organic chemical preparations important to pharmacy in regard to their behaviour to commonly-used reagents. Most of the experiments described were performed in broad daylight, usually at a temperature of from 14° to 20° C.

REVERIES OF THE WORLD'S HISTORY; or, The Romance of a Star. By T. Mullett Ellis. Crown 8vo, pp. viii.—156. (London: Swan Sonnenschein and Co. 1893.) Price 1s.

These reveries, which extend from the earth's nebulous origin to its final ruin, describe:—The Age of Chaos; The First Development and Dawn of Life; The Great Forest Periods; Reptilian Age, Great Animals; Ice Age; The Genesis of Man; Civilisation; Religions; The Present Age; The Doom.

THE ELEMENTS OF NATURAL SCIENCE: A Text-Book for Secondary Schools (in five parts). By Dr. H. Wettstein. Part III., NATURAL PHILOSOPHY. (London: O. Newmann and Co. 1893.) Price 2s. 6d.

A most useful series of books. The one before us presents the subject-matter in such an arrangement that an expansive treatment is possible, and it gives the most needful points of support for thorough progress after oral instruction. It contains 607 illustrations and two coloured plates.

ZOOLOGICAL AND BOTANICAL PLATES.—Messrs. Newmann and Co. have also sent specimens of the above. They are printed in colours on a black ground and measure 40 by 30 inches, and may be had either on canvas, folded or mounted on rollers. We believe there are two sets of Zoological Charts and two of Botanical, each set comprising ten charts. They are very effective.

OBJECT-LESSONS FROM NATURE: A First Book of Science. In two parts. By L. C. Miall, F.R.S. Post 8vo, pp. 240 + 240. (London: Cassell and Co. 1893.) Price 1s. 6d. each.

We have seldom met with books more suitable than these for giving instruction on natural history and general science to very young people. They are nicely illustrated, and the descriptions are given in language which, especially in the first part, we think the youngest child can understand.

THE ART AND PASTIME OF CYCLING. By R. J. Mecredy and G. Stoney. Third edition. Revised by R. J. Mecredy and A. J. Wilson. 8vo, pp. 262. (Dublin: Mecredy and Kyle. London: Iliffe and Son.) Price 2s.

The cyclist will find this a useful book, as it tells him how to keep his machine in good working order, and also instructs him as to the best of the modern types of cycles. The information is simply and plainly given.

DRAWING for Infant Schools. By A. Braithwaite. 4to. (Leeds: E. J. Arnold. London: Simpkin, Marshall, and Co.) Price 1s.

Examples in this book are of the simplest nature, commencing with those suitable for the youngest children in an infant school. It is intended that slates ruled with cross-lines should be used.

THE HUMOUR OF AMERICA. Crown 8vo, pp. xiii.—462. (London: Walter Scott, Limited. 1893.) Price 3s. 6d.

We have here a selection of some of the best writings of Bill Nye, "Orpheus C. Kerr," "Mark Twain," Oliver Wendell Holmes, Will Carlton, and many other of the American humorous writers, together with an Index of American humorists. The illustrations by C. E. Brook are exceedingly good.

COMMON-SENSE EUCLID. Parts I. and II. By Rev. A. D. Capel, M.A. Crown 8vo, pp. 155 + 140. (London: W. H. Allen. 1893.) Price 2s. each.

Part I. comprises Books I. and II., and Part II. Books III. and IV., each with upwards of 300 graduated riders and hints for their solution, the object of the author being to point out to teachers and students those portions of the treatise which either present difficulties to the beginner or perhaps escape their notice altogether. An analysis of the problems is given in every case, and some of the theorems are also worked backwards. We believe these books to be most useful.

PRACTICAL SOLID GEOMETRY. By J. Payne. 12mo, pp. vii.—206 + 48. (London: Thomas Murley.) Price 2s.

This is one of Murby's useful Science and Art Department series of text-books. It is of a twofold character, being at the same time an elementary and an advanced work, forming a complete guide to the study of Projection for Draughtsmen, and is calculated to qualify for a first-class pass in the advanced stage of the Science and Art Department. To this book is added GRAPHIC ARITHMETIC AND STATICS, by J. J. Price, in which will be found much necessary information both for the elementary and advanced stages. We believe a key to the entire work is published.

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AMATEUR PHOTOGRAPHY. By W. J. Lincoln Adams. Fscap. 4to, pp. 90. (New York: The Baker and Taylor Co. 1893.) Price 50c.

A capital little treatise, by one whose experience eminently fits him to prepare a handbook which will serve as a guide to the ordinary work and at the same time introduce the reader to new fields of interest. Besides the instructions usually found in such books, there are papers on Portraiture, Instantaneous, Flash-Light, Orthochromatic, and Composite Photography; Tables, Formulæ, etc.

EVENING WORK for Amateur Photographers. By T. C. Hepworth, F.C.S. Crown 8vo, pp. iv.—196. (London: Hazell, Watson, and Viney. 1890.)

This useful little book describes, among other things, the utility of the glaziers' diamond, the making of lantern-slides by different processes, practical frame-making, enlargements, etc.

AUSFUHRLICHES HANDBUCH der Photographie, Nos. 24 to 33. Von Der. Josef Maria Eder. (Halle: a S. Wilhelm Knapp.)

These ten numbers of Dr. Eder's *Handbook of Photography* are devoted to a description of the various forms of cameras, instantaneous shutters, enlarging apparatus, etc. There are numerous illustrations; some of the plates give examples of work done with the tele-photographic lens, a combination used for photographing distant objects.

RECETTES ET CONSEILS INEDITS A L'AMATEUR PHOTOGRAPHE. Par Georges Jardin. Cr. 8vo, pp. 74. (Paris: Gauthier Villars et Fils. 1893.)

Numerous useful hints and formulæ, hitherto unpublished, are here given for the use of amateur photographers.

CE QU'ON PEUT FAIRE AVEC DES PLAQUES VOILEES. Par Max. Forest. Crown 8vo, pp. 52. (Paris: Gauthier Villars et Fils. 1893.)

In this treatise a number of ingenious methods are suggested for utilising plates that have been fogged by accidental exposure to light.

DAS ATELIER UND LABARATORIUM DES PHOTOGRAPHEN. Large 8vo, pp. 172. By Dr. Josef Maria Eder. (Halle a. S.: Wilhelm Knapp. 1893.)

This is a supplement to Dr. Eder's *Handbook to Photography*. It treats exhaustively of the construction of the studio and its various accessories; the whole is copiously illustrated.

Messrs. Fletcher, Russell, and Co., of Warrington, have sent us a very convenient little Gas-Heater, suitable for boiling a small kettle. It is very artistically enamelled by a patent process. We think microscopical workers will find such an apparatus exceedingly useful for heating water or sand-baths.

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